

**Analysis of Heavy Metal Exposures and Evaluation of Detection Methods for
Heavy Metals in Drinking Water**

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DISSERTATION

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LIST OF ABBREVIATIONS

AAPCC	American Association of Poison Control Centers
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CV	coefficient of variance
FN	false negative
FP	false positive
g	grams
HNO ₃	nitric acid
IARC	International Agency for Research on Cancer
ICP-AES	inductively coupled plasma - atomic emission spectroscopy
ICP-MS	inductively coupled plasma - mass spectrometry
IR	incidence rate
kg	kilogram
MCL	Maximum Contaminant Level
mL	milliliter
NHANES	National Health and Nutritional Examination Survey
NPDS	National Poison Data System
OSHA	Occupational Safety and Health Administration
PEL	Permissible Exposure Limit
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
RfC	reference concentration
RfD	reference dose
SDWA	Safe Drinking Water Act
TN	true negative
TP	true positive
TT	Treatment Technique
µg	microgram
µg/dL	microgram per deciliter
µg/g	microgram per gram
µg/m ³	microgram per cubic meter
µL	microliter
US	United States
US EPA	United States Environmental Protection Agency
US OSHA	United States Occupational Safety and Health Administration
WHO	World Health Organization

SUMMARY

The quality of drinking water worldwide continues to be a major public health issue. In particular, contamination of drinking water by heavy metals is a concerning and continuing problem, even in the United States (US). In the US, the average concentrations for heavy metals in public drinking water supplies are more likely to be at trace levels, with more than 99% of all publicly supplied drinking water 5 parts per billion (ppb) in the US (Agency for Toxic Substances and Disease Registry, 2007b). Lead has been detected in surface waters and groundwater, which supplies drinking water, at ranges from 5 to 30 ppb (Agency for Toxic Substances and Disease Registry, 2007b). Since drinking water contaminated with heavy metals, such as lead, is a major source of exposure to the general population, it is important to accurately detect these contaminants in drinking water. This research involves three aims that enhance our knowledge about the burden of heavy metal exposures in Illinois, and ability to quantify low levels of lead in water.

Aim 1: Examine and characterize the number of cases of heavy metal poisonings in Illinois Hospital Discharge Data

National health survey data is widely used to determine the extent of exposure to a variety of contaminants, including heavy metals. One of the most widely used health surveys is the National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control and Prevention (CDC). This survey is a nationally representative sample of chemical exposures in the US and analyzes biological samples, in conjunction with data obtained from health interviews (Centers for Disease Control and Prevention, 2014a). A second source of surveillance data is from the

American Association of Poison Control Centers (AAPCC) National Poison Data System (NPDS), which collects real-time data on poisonings from a wide range of exposures (American Association of Poison Control Centers, 2015a). However, there are currently no published studies that estimate the number of acute outcomes related to exposure to heavy metals. Lead poisoning is an adverse health outcome from heavy metal exposure that has been well studied in children and in long-term and high-dose exposures in occupational cohorts. Additionally, hospital data has not been used to estimate and characterize acute outcomes to heavy metal exposures in this way. Population-based hospital registry data can be used to determine incidence rates for acute toxicity, though this source cannot be used to detect latency period or chronic outcomes to consider.

From 2010 through 2013, there were 739 heavy metal poisoning cases treated in Illinois hospitals from 2010 through 2013, of which 437 (59%) were outpatient cases and 302 (41%) inpatient cases. Overall, 345 (47%) of all cases had a primary diagnosis of heavy metal poisoning, and 536 (72.5%) cases having a main diagnosis of heavy metal poisoning. Among inpatients cases, the majority (57.9%) had a length of stay of three or more days, with an average length of stay of 5.8 ± 14.5 days and a median length of stay of 3.0 (interquartile range: 2.0, 5.0). For inpatient heavy metal poisoning cases, only 140 (46%) had a routine discharge to their home.

Aim 2: Evaluate the reliability and validity of a new field-based biosensor methodology in comparison with a conventional, laboratory-based detection

methods for cadmium and lead in drinking water across known concentrations in both controlled laboratory setting and environmental samples.

There are limited studies to date that explore the use of field-based biosensor methodology for detection of cadmium and lead in drinking water. Although there is extensive literature evaluating field-based methods for detection of arsenic in drinking water, especially in regions where levels are known to be elevated, few studies have used a metal-specific biosensor system for the detection of multiple contaminants and/or mixture, including heavy metals, in drinking water. Having a reliable, valid and rapid technique that does not require extensive training can potentially be used by local health departments, water authorities, and communities both in the US and internationally. Such technology would allow results to be obtained on-site, within minutes, and at relatively low cost to evaluate public health concerns of heavy metal contamination in drinking water sources. Results could be conveyed to the residents quickly by providing them with health education materials related to the detection method, exposure, and potential health effects. In addition, this biosensor system could be utilized by municipal wastewater treatment plants and industrial sites that discharge into public waterways to ensure that the effluent does not exceed the maximum contaminant level (MCL) or action level treatment technique set by the US Environmental Protection Agency (US EPA).

The field-based fluorimetric biosensor system, ANDalyze, used to quantify known concentrations of cadmium and lead in two different water types showed inconsistent performance. Overall, the method was positively biased for cadmium and negative biased for lead in deionized water. In tap water, the method was unable to detect lead

at concentrations between 5 and 25 ppb. The patterns quantitatively observed in the data were confirmed by statistical analysis. For cadmium, there were no samples measured by the ANDalyze method that were within $\pm 15\%$ of the standard concentration for both deionized water and tap water. For lead in deionized water, 44% (N=8) samples were correctly identified by ANDalyze within $\pm 15\%$ of the standard concentration; however, in tap water, no samples measured by the ANDalyze method that were within $\pm 15\%$ of the standard concentration.

Aim 3: Evaluate the validity and reliability of multiple, commercially available field-based detection methodologies, in comparison to conventional, laboratory-based detection methods for lead in drinking water across known concentrations of lead mixtures in both controlled laboratory setting and environmental samples.

Failing infrastructure, economic hardship, and changes to disinfection processes have created growing concern about the quality of drinking water across the US, especially in the wake of current events of elevated blood lead levels in Flint, MI. There are several mass marketed and commercially available field-based kits for the detection of lead in drinking water that could be readily used by consumers. However, there are no studies in the literature that have tested the validity and reliability of these field-based test kits. There is a need to determine if screening tools like field-based detection kits are providing accurate results to the general public.

The three field-based kits (First Alert Drinking Water Test Kit (First Alert; First Alert Trust, BRK Brands, Inc.; Aurora, IL), WaterSafe Water Test Kit for Lead (WaterSafe; DiscoverTesting.com, Silver Lake Research Corporation; Monrovia, CA),

and Pro-Lab Lead in Water Test Kit (Pro-Lab; Professional Laboratories; Weston, FL)) were evaluated for the detection of lead and lead mixtures in drinking water. The three kits varied in performance when lead was present alone or in mixtures and water type. Overall, for all three test kits, the performance for lead mixtures in tap water were highly variable, with the First Alert and WaterSafe kits resulting in mostly all FNs and 64% of the Pro-Lab measured lead concentrations in tap water being outside a $\pm 15\%$ accuracy range. For lead only standards in tap water, FN rate was 64%, the sensitivity was 29%, and accuracy was 67%, and precision was 100% for First Alert. However, for lead mixtures in tap water, the FN rate was 90%, sensitivity was 10%, and accuracy was 20% for First Alert. For the lead only standards in tap water, the FN rate was 40%, sensitivity was 60%, and accuracy was 79% for WaterSafe. For the mixtures of lead standards in tap water, the FN rate was 75%, sensitivity was 25%, and accuracy was 40% for WaterSafe. The ANOVA analysis found that there were statistically significant differences between the Pro-Lab measured concentrations and the standard concentrations ($p=0.05$), water type ($p=0.002$), and the interaction of these two variables ($p=0.01$), with and $R^2=0.89$.

1. BACKGROUND

One of the most basic necessities to human health is safe drinking water. Water quality, sanitation, and water quantity are challenges faced by both developed and developing countries. In particular, heavy metal contamination in drinking water continues to be a public health issue worldwide. In both rural and urban settings, heavy metals have become ubiquitous in the environment. Low level exposures to heavy metals such as cadmium and lead are thought to be associated with a myriad of health effects, ranging from neurodevelopmental to renal effects and even cancer (Agency for Toxic Substances and Disease Registry, 1992; 1999; 2002; 2007a; 2007b; 2012a; 2012b; 2012c; 2013). These low level exposures to heavy metals occur in part through contaminated drinking water in the US and worldwide.

Challenges exist in identifying and measuring contaminants in drinking water supplies due to their presence at low concentrations and in mixtures (Villanueva et al., 2014). The identification of specific contaminants and quantification of detection limits may not be as accurate when chemicals are measured in a mixture rather than in isolation (Altenburger et al., 2015; Brack et al., 2015). Furthermore, current detection methods for testing heavy metals in water typically involve transporting samples from the field to a laboratory, where analysis is carried out on complex analytical instruments by trained personnel: This scenario is relatively time and labor intensive, and each analytical instrument has unique limitations with respect to sensitivity and/or specificity (Raja and Selvam, 2011; Rategarpanah et al., 2013). Current detection methods used to quantify heavy metals in drinking water include inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectroscopy (ICP-

AES) cold vapor atomic absorption spectrometry, and UV visible spectrometry, (Raja and Selvam, 2011; Rategarpanah et al., 2013). New analytical methods could provide data in the field, and at lower cost, which would enrich options for exposure assessment of heavy metals in drinking water.

1.1 Exposure to Heavy Metals

Heavy metals are naturally occurring elements that have been used for thousands of years in various medical and industrial activities (Järup, 2003). Although some metals are essential for human health, such as iron and zinc, exposure to these metals at high levels, and to non-essential metals (like arsenic, cadmium, lead, and mercury), remain major threats to human health (Järup, 2003). Although adverse health effects of high exposure to heavy metals have been well studied, exposure to heavy metals continues, albeit emissions from industrial sources have declined, especially in the last several decades (Järup, 2003). The World Health Organization (WHO) has stated that lead, mercury, and cadmium are among the top ten more dangerous chemicals to human health when present in drinking water (Cerminati et al., 2015).

Heavy metal exposures are typically greater in the occupational setting than in the ambient environment and this disparity is codified in federal occupational and environmental health regulations and policies (Agency for Toxic Substances and Disease Registry, 1992; 1999; 2002; 2007a; 2007b; 2012a; 2012b; 2012c; 2013), see Table I for selected occupational exposure limits. In the occupational setting, there is increased potential for acute, high-dose exposures, as well as for chronic exposure. Industrial processes such as smelting and mining are major concerns for worker health

and safety, with respect to heavy metals, including arsenic, beryllium, cadmium, lead, and mercury (Occupational Safety and Health Administration, 2016; Agency for Toxic Substances and Disease Registry, 2007b; Agency for Toxic Substances and Disease Registry, 2012; Occupational Safety and Health Administration, 2016). Epidemiological studies of occupational cohorts have provided key insights into adverse health outcomes associated with heavy metal exposures, but the relatively high exposures in occupational setting limit interference to the general public.

TABLE I
OCCUPATIONAL REGULATIONS FOR EXPOSURE TO HEAVY METALS

OSHA Permissible Exposure Limit	8-hour Time Weighted Average (mg/m³)
Antimony	0.500
Arsenic	0.010
Beryllium	0.002
Cadmium	0.005
Chromium (Metal and Insoluble Salt)	1.0
Lead (Inorganic)	0.050
Manganese	5.0
Mercury (as vapor)	0.100

Exposure to heavy metals in the general US population is widespread, but typically involves low doses. Contaminated air, soil, drinking water, and food are the common mediums of exposure to heavy metals such as arsenic, lead, and mercury. Heavy metal exposures in the general US population is reported in NHANES. NHANES is a nationally representative sample of the general population and shows that the general population is exposed to a multitude of chemicals, including heavy metals (Centers for Disease Control and Prevention, 2014a). Low (trace) levels of heavy

metals have been recorded in biological specimens in the NHANES data over the last several survey periods. Table II provides a summary of the biological measures of heavy metals from NHANES and these are within available clinical reference values shown in Table III (Centers for Disease Control and Prevention, 2014a; Mayo Clinic, 2016).

TABLE II
SUMMARY OF BIOLOGICAL MEASURES OF HEAVY METALS FROM NHANES

Metal	Urinary^a	Blood	Survey Year
Antimony	0.06 (0.06, 0.06) µg/g	-	2009-2010
Arsenic, total	7.77 (6.85, 8.81) µg/g	-	2011-2012
Cadmium	0.18 (0.17, 0.19) µg/g	0.28 (0.27, 0.29) µg/L	2011-2012
Lead	0.41 (0.38, 0.44) µg/g	0.97 (0.92, 1.04) µg/dL	2011-2012
Manganese	0.14 (0.13, 0.16) µg/g	9.35 (9.19, 9.51) µg/L	2011-2012
Mercury, total	0.36 (0.33, 0.41) µg/g	0.70 (0.62, 0.80) µg/L	2011-2012

^aCreatinine adjusted

TABLE III
CLINICAL REFERENCE VALUES FOR SELECTED HEAVY METALS IN URINE

Metal	Reference Value (µg/L)
Arsenic	0-35
Cadmium	
0-15 year	Not established
16 years or older	0.0-1.3
Lead	0-4
Mercury	
0-15 year	Not established
16 years or older	0-9
Toxic Concentration	>50

1.2 US Drinking Water Quality Standards

Under the Safe Drinking Water Act (SDWA), the US EPA is required to determine the level of certain contaminants, like heavy metals, in drinking water at which no adverse health effects are likely to occur (United States Environmental Protection Agency, 2014a). The National Primary Drinking Water Regulations, which result from the US EPA analysis, can include maximum contaminant levels (MCLs). MCLs are legally enforceable standards that define a target contaminant concentration in finished drinking water. The MCL is developed with consideration of possible health risks associated with a lifetime exposure and an adequate margin of safety, costs, benefits, and the ability of public water systems to detect and remove contaminants using suitable treatment technologies (United States Environmental Protection Agency, 2013a; United States Environmental Protection Agency, 2014b). For cadmium, the MCL is 5 ppb (0.005 ppm).

In the absence of an economical or technological reliable method to measure contaminants at low concentrations, a treatment technique (TT) action level is set instead of an MCL (United States Environmental Protection Agency, 2013a). Like an MCL, a TT action level is an enforceable level of technological performance that public water systems must meet to ensure control of contaminants (United States Environmental Protection Agency, 2013a). In 1991, under the SDWA, US EPA published a new regulation regarding the control of lead and copper in drinking water, the Lead and Copper Rule (United States Environmental Protection Agency, 2014d). The Lead and Copper Rule was promulgated to help “minimize waterborne lead exposure through the identification and control of corrosion, which is the primary

mechanism for lead release in municipal drinking water systems” (Pieper et al., 2015). Under this regulation, the MCL was replaced with a TT action level of 15 ppb for lead and 1300 ppb for copper (United States Environmental Protection Agency, 2014c).

In addition to Primary Drinking Water Regulations, US EPA also sets Secondary Drinking Water Regulations. These Secondary Drinking Water Regulations are non-mandatory water quality standards for 15 contaminants that were established as recommendations for drinking water aesthetic (tastes or odors), cosmetic (effects which do not damage the body, but are undesirable), and technical (damage to water equipment or reduced effectiveness of treatment for other contaminants) effects (United State Environmental Protection Agency, 2016a). The Secondary Drinking Water Regulation for iron is 0.3 ppm (United State Environmental Protection Agency, 2016a).

1.3 Current Issues of Lead in Drinking Water

Although drinking water is often regarded as a trivial source of lead exposure among the general population, it has been estimated that tap water once contributed to as much as 10-20% of total lead exposure in the US (National Toxicology Program, 2012). In addition, people in specific locations at specific times may have high exposure to lead in drinking water due to failures of public infrastructure management. For example, the identification of elevated blood lead levels in children in Flint, MI, which has been associated with lead exposure through drinking water that began with a change in the source and treatment of drinking water for the city, has drawn national attention to heavy metals exposure in drinking water (Bellinger, 2016; Sanburn, 2016). Unfortunately, the crisis in Flint is just one of many in recent decades (Bryant, 2004;

Guidotti et al., 2007; Miranda et al., 2007; Renner, 2009; Milman and Glenza, 2016; Wood, 2016; Wood and Ruderman, 2016). As infrastructure continues to age in many cities and towns across the US, potential modifications in treatment techniques, and changes in source water, the problems seen in Flint are likely to happen elsewhere.

1.3.1 Philadelphia, PA

Water testing procedures and sampling problems have plagued Philadelphia in recent decades. Between May 2000 and January 2001, 292 Philadelphia schools were tested for lead in drinking water (Bryant, 2004). This analysis found that 57.4% of the sampled schools had water with greater than 20 ppb lead (US EPA's TT action level is 15 ppb), and 28.7% of those schools had a mean lead concentration in water in excess of 50 ppb (Bryant, 2004). The most recently published water quality report for Philadelphia reports testing of customers' taps for lead and copper in only 134 homes every three years – the federal standard (Philadelphia Water, 2015). Furthermore, recent news articles have pointed to the inadequacies of the how homes are selected for sampling, how often they are sampled, and the procedures for collecting water samples, which may reduce the amount of lead in the sample (Milman and Glenza, 2016; Wood, 2016; Wood and Ruderman, 2016).

1.3.2 Washington, DC

In 2001, there was an abrupt increase in lead concentrations in drinking water in Washington, DC (Guidotti et al., 2007; Wines and Schwartz, 2016). The change from chlorine as the disinfection treatment to chloramine altered the water chemistry, and

increased leaching of lead from pipes into the water (Renner, 2009). One mitigation strategy in this scenario is to replace lead water service lines, but this may not decrease the risk of elevated blood lead levels (Brown et al., 2011).

1.3.3 Chicago, IL

As required by federal regulations, the City of Chicago tests drinking water for lead in 50 homes every three years, but there is an estimated 2.7 million homes built prior to 1986 that could have lead water service pipes (Editorial Board, 2016). Of the 50 homes tested in the last sampling cycle, only three were located where the water main had been replaced during the past five years (Hawthorne and Smith Richards, 2016a). While this sampling methodology and testing does meet the federal law, it is still drawing criticism because 80% of Chicago properties have lead service lines (Hawthorne and Smith Richards, 2016a).

Federal regulations do not require schools to routinely test drinking water, but in wake of these controversies about water testing in private residences, Chicago Public Schools (CPS) revealed that drinking water in schools had not been test for lead in recent years, and began testing schools in April 2016 (Hawthorne and Smith Richards, 2016b; Perez Jr., 2016a). Preliminary tests found at least 11 of 30 (37%) schools sampled had lead levels in water above the US EPA TT action level of 15 ppb (Perez Jr., 2016b).

1.3.4 Flint, MI

Flint, MI was once a pivotal manufacturing center for General Motors (GM), but with the decline in auto manufacturing and sales in recent years, the local economy has fallen into economic hardship (Sanburn, 2016). As a cost-saving measure, the city council voted to stop purchasing water from Detroit, and instead use a new regional pipeline, Karegnondi Water Authority that would, like Detroit, draw water directly from Lake Huron (Sanburn, 2016). The projected savings was estimated to be \$19 million over eight years (Sanburn, 2016). However, a major problem arose from this decision: construction for the new pipeline had not even started before the City of Detroit retaliated and stopped selling water to Flint (Sanburn, 2016).

The short-term solution while waiting for the new pipeline to be built was to have the City of Flint draw water from the Flint River (Bellinger, 2016; Sanburn, 2016). Unfortunately, corrosion control measures necessary to mitigate high levels of chlorine in the Flint River water and the addition of ferric chloride (used to reduce the formation of trihalomethanes from organic matter during treatment), were not applied (Bellinger, 2016; Sanburn, 2016).

Following the switch to the Flint River, residents noticed discoloration, odor, and difference in taste (Sanburn, 2016). Within four months of the switch, the City detected high levels of *E. coli* in the water and residents were ordered to boil their water (Sanburn, 2016). Six months after the switch, GM announced that they would stop using local water at one of their plants, as the water was corroding engine parts (Sanburn, 2016). Soon, residents were asking for their water to be tested, and in six of nine city wards, water in over 20% of homes tested had lead concentrations greater

than 15 ppb, the US EPA's TT action level; some samples were in excess of 100 ppb (Bellinger, 2015; United States Environmental Protection Agency, 2014d). Even after the *E. coli* issues, GM's announcement, and residents' concerns, the city did not switch back to the Detroit water due to financial concerns (Sanburn, 2016). The city maintained that the water was safe and that these were isolated incidents of high lead levels and not part of a systematic problem (Sanburn, 2016).

Not until October 16, 2015, nearly two and half years after changing the supply to the Flint River, did the City of Flint finally decide to switch back to Detroit water. However, the damage to the pipes and increased blood lead levels had already occurred. A recent study examining blood lead levels in children before and after the change in drinking water sources in Flint found that the incidence of elevated blood lead levels increased from 2.4% to 4.5% ($p < 0.05$) (Hanna-Attisha, et al., 2016). In 2014, of children under 72 months of age, there was an estimated 0.53% and 0.46% of children tested that had confirmed blood lead levels greater than 10 $\mu\text{g/dL}$ in the US and in Michigan, respectively (Centers for Disease Control and Prevention, 2016a). Additionally, in neighborhoods that had the highest concentrations of lead in drinking water, there was a 6.6% increase in the incidence of elevated blood lead levels and, that the greatest elevated blood lead level increases were in neighborhoods identified as (economically) disadvantaged (Hanna-Attisha, et al., 2016).

Since the news broke about the problems in Flint, other incidents from across the US have emerged, including Sebring, OH, Philadelphia, PA, Boston, MA and Chicago, IL (Milman and Glenza, 2016). Though these incidents are not quite to the scale of Flint, they reflect that lead exposures through drinking water are an important public

health challenge. The issues in Flint and in other parts of the US have caused public health and environmental agencies at all levels of government to reevaluate the way that drinking water testing is done, how best to address inadequacies in testing, and how to establish prevention measures to ensure that the crisis in Flint does not happen again.

2. LITERATURE REVIEW

2.1 Overview of Heavy Metals

Heavy metals are ubiquitous in the environment, as they are both naturally occurring and from anthropogenic sources. A brief overview of the routes of exposure, adverse health outcomes, and comparison to national health survey data is provided for antimony, arsenic, beryllium, chromium, manganese, and mercury, following with a more detailed review of cadmium and lead. These metals were chosen because of the availability of diagnosis codes (NCOES and ECODES) in the hospital discharge data. Cadmium and lead were chosen for the drinking water field kit testing because of the gaps in literature about the reliability of field kits that detect these metals.

2.1.1 Antimony

Antimony is naturally occurring in the earth's crust and is silver-white metal (Agency for Toxic Substances and Disease Registry, 1992). Antimony enters the environment through the mining process; however, little or no antimony is mined in the US (Agency for Toxic Substances and Disease Registry, 1992). Once released into the environment, antimony can stay in the air for days, but majority ends up in the soil, where it binds with iron, manganese, or aluminum (Agency for Toxic Substances and Disease Registry, 1992).

Since antimony is naturally occurring in the environment, the general population is exposed at low levels, primarily in food, water, and air (Agency for Toxic Substances and Disease Registry, 1992), but those that work in mining, smelters, or other industries that process or use antimony ore may be exposed to higher levels (Agency for Toxic

Substances and Disease Registry, 1992). Concentrations at high levels of 9 milligrams per cubic meter (mg/m^3) of antimony can cause irritation to eyes, skin, and lungs (Agency for Toxic Substances and Disease Registry, 1992). However, breathing concentration as low as 2000 ($\mu\text{g}/\text{m}^3$) can cause respiratory (pneumoconiosis), cardiovascular (altered electrocardiogram), gastrointestinal (abdominal pains, diarrhea, vomiting, and stomach ulcers) effects (Agency for Toxic Substances and Disease Registry, 1992). After antimony enters the body, it is eliminated over several weeks (Agency for Toxic Substances and Disease Registry, 1992).

Because of potential adverse health effects, the US EPA set the MCL of 0.006 ppm in drinking water, with a reference dose (RfD) of 0.0004 milligrams per kilograms per day ($\text{mg}/\text{kg}/\text{day}$) (United States Environmental Protection Agency, 2014a; United States Environmental Protection Agency, 2014d). To protect worker health and safety, the US Occupational Safety and Health Administration (US OSHA) set the permissible exposure limit (PEL) at 0.5 mg/m^3 for an 8-hour time weighted average (Agency for Toxic Substances and Disease Registry, 1992).

2.1.2 Arsenic

Arsenic is a naturally occurring element, but also has been used in industrial processes (Agency for Toxic Substances and Disease Registry, 2007a). Most inorganic and organic arsenic compounds are white or colorless powders that are odorless, tasteless, and do not evaporate (Agency for Toxic Substances and Disease Registry, 2007a). Arsenic was once largely used in industrial processes, such as mining or smelting, and as pesticides, but it is no longer produced in the US (Agency for Toxic

Substances and Disease Registry, 2007a). Currently, the large majority of arsenic (approximately 90%) is used as a wood preservative (Agency for Toxic Substances and Disease Registry, 2007a).

Whether released into the environment naturally or through anthropogenic sources, arsenic can be found in air, water, soil, and food (Agency for Toxic Substances and Disease Registry, 2007a). In soil, the average concentrations of arsenic is 3-4 ppm and in air, depending on location, the range from 1 to 2000 nanograms (ng) (Agency for Toxic Substances and Disease Registry, 2007a). However, drinking water is the major route of exposure to arsenic, with approximately 80% of US drinking water supplies having less than 2 ppb of arsenic; however, 2% have been found with an excess of 20 ppb (Agency for Toxic Substances and Disease Registry, 2007a).

Potential adverse health effects depend on the amount that was inhaled, ingested or dermally absorbed, although dermal exposure is of lesser concern (Agency for Toxic Substances and Disease Registry, 2007a). The most common form of arsenic that the general population is exposed to is inorganic arsenic via ingestion of contaminated drinking water or from contaminated food (Agency for Toxic Substances and Disease Registry, 2007a). Once ingested, inorganic arsenic enters the gastrointestinal tract, and becomes absorbed (95% absorption) (Agency for Toxic Substances and Disease Registry, 2007a). Arsenic concentrations measured in hair (0.65 ppm) and nails (0.36ppm) were slightly higher than concentration levels found in tissues (0.05-0.15 ppm), indicating that there is little affinity for arsenic to accumulate preferentially in any internal organs (Agency for Toxic Substances and Disease Registry, 2007a). The primary target organ for arsenic is the skin; however, other target

organs include kidneys, liver, heart, and nervous system (Agency for Toxic Substances and Disease Registry, 2007a). Cancer of the skin, kidneys and liver, in addition to peripheral vascular effects, cardiovascular effects including increased incidences of high blood pressure and circulatory problems are all suspected health outcomes of long-term arsenic exposure (Agency for Toxic Substances and Disease Registry, 2007a). However, the most commonly recognized sensitive endpoint is hyperpigmentation and hyperkeratosis of the skin (Agency for Toxic Substances and Disease Registry, 2007a; United States Environmental Protection Agency, 2014c). Furthermore, the International Agency for Research on Cancer (IARC) has classified arsenic as Group 1 carcinogenic (carcinogenic to humans) (Agency for Toxic Substances and Disease Registry, 2007a).

Because of these potential adverse health effects associated with exposure to arsenic, the US EPA has set the drinking water MCL of 0.01 ppm, with an established RfD for arsenic of 0.0003 mg/kg/day (Agency for Toxic Substances and Disease Registry, 2007a; United States Environmental Protection Agency, 2014a; United States Environmental Protection Agency, 2014e). OSHA has also set a PEL of 10 micrograms per cubic meter ($\mu\text{g}/\text{m}^3$) for an 8-hour time weighted average (Agency for Toxic Substances and Disease Registry, 2007a).

2.1.3 Beryllium

Beryllium is a naturally occurring element that has been found in coal, oil, soil, and volcanic dust (Agency for Toxic Substances and Disease Registry, 2002). Beryllium can be blue-green or green color and is soluble in water (Agency for Toxic

Substances and Disease Registry, 2002). Most beryllium and its compounds are mined and converted to alloys for electrical and electronic parts or construction materials for plastic molds and machinery; however, beryllium alloys have been used in computers, sports equipment (golf clubs and bike frames), cars, and dental bridges (Agency for Toxic Substances and Disease Registry, 2002).

Beryllium can enter the environment from both natural and anthropogenic sources (Agency for Toxic Substances and Disease Registry, 2002). In the US, the average concentration of beryllium in air is 0.03 ng/m^3 , with fine beryllium particles remaining in the air for approximately 10 day (Agency for Toxic Substances and Disease Registry, 2002). Beryllium can be found in varying amounts in drinking water, with US EPA reporting that less than two trillionth of a gram per liter of water (Agency for Toxic Substances and Disease Registry, 2002). These concentrations may be higher if living within close proximity of an industry that processes or use beryllium or its compounds (Agency for Toxic Substances and Disease Registry, 2002).

For the general population, beryllium enters the body through inhalation or ingestion, as beryllium is poorly absorbed dermally (Agency for Toxic Substances and Disease Registry, 2002). If beryllium is ingested, less than 1% passes from the stomach and intestines to the bloodstream; thus, most of the beryllium ingested is excreted from the body within days and without entering the bloodstream (Agency for Toxic Substances and Disease Registry, 2002). However, if beryllium is inhaled, it may stay within the body for months to years (Agency for Toxic Substances and Disease Registry, 2002). Breathing greater than 1 mg/m^3 of beryllium can cause acute beryllium disease, which is lung damage representing pneumonia with reddening and swelling of

the lungs (Agency for Toxic Substances and Disease Registry, 2002). In occupational settings and communities with continuous exposure to ambient air concentrations of beryllium, hypersensitivity or allergy can develop, resulting in chronic beryllium disease (Agency for Toxic Substances and Disease Registry, 2002). Chronic beryllium disease happens after sensitivity to beryllium occurs, causing immune or inflammatory reactions if exposed to small amounts of beryllium and white cells will accumulate around the inhaled beryllium and form a chronic inflammatory reaction (granulomas) (Agency for Toxic Substances and Disease Registry, 2002). Chronic beryllium disease may occur 10-15 years after exposure (Agency for Toxic Substances and Disease Registry, 2002). In addition to respiratory effects, beryllium can cause ulcers and skin granulomas (from dermal contact). IARC has classified beryllium as a Group 1 carcinogen.

US EPA has set the MCL for beryllium in drinking water at 0.004 ppm, a RfD of 0.002 mg/kg/day, and a reference concentration for inhalation exposure (RfC) of 0.00002 mg/m³ (United States Environmental Protection Agency, 2014a; United States Environmental Protection Agency, 2014f). The 8-hour time weighted average set by US OSHA is 2 µg/m³, with the National Institute of Occupational Health and Safety (NIOSH) recommending 0.5 µg/m³ for an 8-hour work shift (Agency for Toxic Substances and Disease Registry, 2002).

2.1.4 Chromium

Chromium is a naturally occurring element that is tasteless and odorless (Agency for Toxic Substances and Disease Registry, 2012a). There are three common forms of chromium: chromium (0), chromium (III), and chromium (VI) (Agency for Toxic

Substances and Disease Registry, 2012a). Chromium (0) is commonly used for making steel, while chromium (III) and chromium (VI) are used in chrome plating, dyes and pigments, leather tanning, and preserving wood (Agency for Toxic Substances and Disease Registry, 2012a). If released from industries that manufacture or use chromium products, it can be found in air, water, soil (Agency for Toxic Substances and Disease Registry, 2012a). Chromium does not remain in air for long (less than ten days) and is usually deposited into soil and water, where it can easily change from one form to another, depending on the conditions (Agency for Toxic Substances and Disease Registry, 2012a). It is estimated that approximately one third of atmospheric releases of chromium are in the form of chromium (VI) (Agency for Toxic Substances and Disease Registry, 2012a). The median ambient air concentrations for chromium is 20 ng/m³, with indoor air concentrations 10-400 times higher with the presence of cigarette smoke (Agency for Toxic Substances and Disease Registry, 2012a). The average concentration of chromium in soil is 37 ppm, while drinking water contains less than 5 ppb of chromium (Agency for Toxic Substances and Disease Registry, 2012a).

Ingestion and inhalation are the main routes of exposure for all forms of chromium, with dermal absorption a minor route of exposure for the general population (Agency for Toxic Substances and Disease Registry, 2012a). Chromium (III) is an essential nutrient and helps the body use fat, protein, and sugar in the human body and do not appear to cause health problems (Agency for Toxic Substances and Disease Registry, 2012a). However, chromium (VI) can cause adverse health effects: if high levels are inhaled, it can cause irritation to the nose, nose ulcers, and respiratory problems such as asthma and wheezing (Agency for Toxic Substances and Disease

Registry, 2012a). In addition to respiratory effects, chromium (VI) has been associated with gastrointestinal (irritation, ulcers, and lesions in the stomach and small intestine after ingestion), hematological (microcytic, hypochromic anemia), and reproductive (decreased sperm count and epididymal damage) effects (Agency for Toxic Substances and Disease Registry, 2012a). Chromium (VI) has been classified as a Group 1 carcinogen by IARC, while chromium (III) is not considered carcinogenic to humans (Agency for Toxic Substances and Disease Registry, 2012a).

The MCL for total chromium is 0.1 ppm, with an RfD of 0.003 mg/kg/day, and RfC of 8×10^{-6} mg/m³ for dissolved chromium (VI) aerosols and 0.0001 mg/m³ for chromium (VI) particulates (United States Environmental Protection Agency, 2014a; United States Environmental Protection Agency, 2014g). The PEL is 1 mg/m³ for an 8-hour work day, while NIOSH recommends 0.5 mg/m³ for an 8-hour work day (Occupational Health and Safety Administration, 2012a).

2.1.5 Manganese

Manganese is a naturally occurring element that is mainly found as oxides, carbonates, or silicates (Agency for Toxic Substances and Disease Registry, 2012b). Manganese has largely used in steel production to improve hardness, stiffness, and strength, but inorganic manganese has been used in the production of batteries, glass, fireworks, leather textiles, and fertilizer, while organic manganese (methylcyclopentadienyl manganese) is added to gasoline to improve the anti-knock properties of fuel (Agency for Toxic Substances and Disease Registry, 2012b). Manganese can be released into the environment through natural and anthropogenic

sources, including being released from iron and steel production facilities and power plants (Agency for Toxic Substances and Disease Registry, 2012b).

For the general population, inhalation and ingestion are the main routes of exposures to manganese, but for occupational cohorts, inhalation is the major route of exposure (Agency for Toxic Substances and Disease Registry, 2012b). The estimated average air concentrations in urban areas is 40 ng/m³ and less than 10 ng/m³ in rural areas (Agency for Toxic Substances and Disease Registry, 2012b). In water, the median manganese concentration is 10 ppb (Agency for Toxic Substances and Disease Registry, 2012b). For soil, the average concentration of manganese is 330 ppm (Agency for Toxic Substances and Disease Registry, 2012b).

Manganese is an essential nutrient, but at high concentrations, can cause adverse health outcomes (Agency for Toxic Substances and Disease Registry, 2012b). If manganese is inhaled, it can be transported directly to the brain, resulting in manganism, a permanent neurological disorder that can cause tremors, difficulty walking and facial muscle spasms (Agency for Toxic Substances and Disease Registry, 2012b). At air high concentrations, manganese can also cause lung irritation and reproductive effects, if inhaled (Agency for Toxic Substances and Disease Registry, 2012b). Ingestion of manganese has also been associated with neurological effects (Agency for Toxic Substances and Disease Registry, 2012b).

Because manganese is an essential element for human health, there is no MCL set, but rather, a Secondary Drinking Water Regulation of 0.05 ppm, as manganese can cause brown-black color, black staining, and/or bitter metallic taste (United States Environmental Protection Agency, 2016a). The RfD for manganese is 0.14 mg/kg/day

and the RfC is 5×10^{-5} mg/m³ (United States Environmental Protection Agency, 2014h). The US OSHA PEL is 5 mg/m³, but NIOSH has a recommended exposure limit for an 8-hour time weighted average as 1 mg/m³ (Occupational Health and Safety Administration, 2004).

2.1.6 Mercury

Mercury is a naturally occurring metal and has multiple forms, including elemental and metallic (Agency for Toxic Substances and Disease Registry, 1999). Metallic mercury is shiny, silver-white liquid that is odorless (Agency for Toxic Substances and Disease Registry, 1999). Mercury can combine with elements like chlorine or oxygen to form inorganic mercury compounds (mercury salts), which are white powders, or mercury can combine with carbon to form organic mercury compounds, such as methylmercury (Agency for Toxic Substances and Disease Registry, 1999). Metallic mercury has been used in thermometers, dental fillings and batteries (Agency for Toxic Substances and Disease Registry, 1999). Mercury salts have been used in skin lightening creams (Agency for Toxic Substances and Disease Registry, 1999).

Approximately 80% of inorganic mercury released into the air is from anthropogenic sources as emissions from mining ore deposits, coal burning and waste, and manufacturing plants, but is released into water and soil from natural deposits and waste disposal (Agency for Toxic Substances and Disease Registry, 1999). Between 10 and 20 ng/m³ of mercury has been measured in outdoor air in urban areas (Agency for Toxic Substances and Disease Registry, 1999). Methylmercury is formed in water

and sediment by bacteria, and bioaccumulates through the aquatic food chain (Agency for Toxic Substances and Disease Registry, 1999). Mercury concentrations in surface water are typically less than 5 ppt (Agency for Toxic Substances and Disease Registry, 1999). In soil, mercury concentrations range from 20 – 625 ppb (Agency for Toxic Substances and Disease Registry, 1999). For the general population, consumption of contaminated fish or shellfish is the major route of exposure to methylmercury, with inhalation of vapors from spills, incinerators, and industries that burn mercury-containing fossil fuels (Agency for Toxic Substances and Disease Registry, 1999).

The nervous system is the target organ for methylmercury and metallic mercury vapors (Agency for Toxic Substances and Disease Registry, 1999; Agency for Toxic Substances and Disease Registry, 2013). Mercury vapors from methylmercury and metallic mercury can breach the blood-brain barrier, resulting in neurological effects (Agency for Toxic Substances and Disease Registry, 1999; Agency for Toxic Substances and Disease Registry, 2013). Mercury can also result in permanent damage to the neurocognitive functions (irritability, shyness, tremors, changes in vision or hearing, and memory problems), kidney effects and damage to developing fetus (Agency for Toxic Substances and Disease Registry, 1999; Agency for Toxic Substances and Disease Registry, 2013). Acute exposure to high levels to metallic mercury may result in lung damage, nausea, vomiting, diarrhea, increased blood pressure and/or heart rate, skin rashes, and eye irritation (Agency for Toxic Substances and Disease Registry, 1999; Agency for Toxic Substances and Disease Registry, 2013).

As a result of these potential severe adverse health effects, the RfC for mercury is 0.0003 mg/m³ and the MCL is 0.002 ppm (United States Environmental Protection

Agency, 2014i; United States Environmental Protection Agency, 2014a). For metallic mercury, the PEL is 0.1 mg/m³ for an 8-hour time-weighted average, while the NIOSH recommended 8-hour time weighted average is 0.05 mg/m³ (Occupational Health and Safety Administration, 2012b).

2.2 Cadmium

Cadmium is a naturally occurring metal within the earth's crust, and is a known human carcinogen, with non-essential, non-beneficial, and highly toxic potential even at low doses to humans and aquatic life (Shing et al., 2008). Cadmium is widely used in several industrial processes including battery production, pigment production and use, plastics production, metal mining, and smelting and refining (Agency for Toxic Substances and Disease Registry, 2012c; World Health Organization, 2011). From these processes, cadmium is emitted into air, water, and soil. In unpolluted natural waters, cadmium concentrations are typically below 1 ppb and between 5,000-100,000 ppt in ocean water (Agency for Toxic Substances and Disease Registry, 2012c; World Health Organization, 2011). The concentration of cadmium in drinking water sources can be influenced by pH, with a low pH and soft waters reporting higher cadmium levels (World Health Organization, 2011). In addition, cadmium exists in water as the hydrated ion or as ionic complexes with other inorganic or organic substances (Agency for Toxic Substances and Disease Registry, 2012c). The soluble forms migrate in water, while the insoluble forms become immobile, are deposited, and get absorbed into sediments (Agency for Toxic Substances and Disease Registry, 2012c). Although cadmium levels are expected to be low in drinking water and ambient air, within the

vicinity of cadmium-emitting industries or incinerators, this expectation does not hold true. Water sources near cadmium-emitting industries, both historic and current operations, have shown elevated concentrations of cadmium in water sediments and aquatic organisms, with a concentration ranging from less than 1 ppb to 77 ppb (Agency for Toxic Substances and Disease Registry, 2012c). In NHANES survey year 2011-2012, the geometric mean for blood cadmium levels was 0.279 micrograms per liter ($\mu\text{g/L}$) (95% CI: 0.267, 0.292), based on a sample size of 7,920 in survey year 2011-2012 (Centers for Disease Control and Prevention, 2015a). For the same survey years, the reported geometric mean for urinary cadmium, creatinine corrected, was 0.176 $\mu\text{g/g}$ creatinine (95% CI: 0.165, 0.187) (Centers for Disease Control and Prevention, 2015a). However, NHANES does not provide reference guidance to put these measured values into context.

2.2.1 Routes of Exposure for Cadmium

The main route of exposure to cadmium in the general non-smoking population is through contaminated food and drinking water (Agency for Toxic Substances and Disease Registry, 2012c; United Nations Environment Programme, 2008). It is estimated that the average weekly intake of cadmium from food ranges from 0.7 micrograms per kilogram ($\mu\text{g/kg}$) of body weight to 2.8 $\mu\text{g/kg}$ body weight (United Nations Environment Programme, 2008). For smokers, the main route of exposure to cadmium is through inhalation of smoke, with ingestion of contaminated water and food as the secondary source (Agency for Toxic Substances and Disease Registry, 2012c;

National Toxicology Program, 2014). Smokers are exposed to approximately 1.7 μg of cadmium per cigarette (National Toxicology Program, 2014).

The highest risk of occupational exposure to cadmium is for individuals who work in industries that heat cadmium-based materials, such as smelting and electroplating, and in manufacturing of batteries (Agency for Toxic Substances and Disease Registry, 2012c; Thévenold and Lee, 2013). The major route of occupational exposure to cadmium is through inhalation of fumes and dust from industrial processes, with incidental ingestion from contaminated hands, food and water, and from smoking (Agency for Toxic Substances and Disease Registry, 2012c; Thévenold and Lee, 2013). Ambient air concentrations near lead and zinc smelters have been found in levels up to 1.2 micrograms per cubic meter ($\mu\text{g}/\text{m}^3$) (Agency for Toxic Substances and Disease Registry, 2012c; Thévenold and Lee, 2013). The OSHA PEL, the time-weighted average concentration that must not be exceeded during any 8-hour work shift in a 40-hour work week, as 5 $\mu\text{g}/\text{m}^3$ for all cadmium compounds, dust, and fumes (Occupational Safety and Health Administration, 2003).

Once cadmium enters the body, whether ingested or inhaled, cadmium is widely distributed throughout the body, with accumulation in the kidneys and liver (Agency for Toxic Substances and Disease Registry, 2012c; World Health Organization, 2011a; World Health Organization, 2011b). Cadmium does not undergo metabolic biotransformation such as oxidation, reduction or alkylation (Agency for Toxic Substances and Disease Registry, 2012c). Absorbed cadmium is excreted extremely slowly, with a half-life of 10-35 years (Agency for Toxic Substances and Disease Registry, 2012c). The target organ for cadmium from oral exposure is the kidney, with

bone as an additional sensitive target, and with adverse effects of renal tubular damage, glomerular damage, decreased bone mineralization, and increased risk for bone fractures (Agency for Toxic Substances and Disease Registry, 2012c). For those that are exposed occupationally to cadmium, the target organ is the lungs; however, renal and bone effects are also major target endpoints (Agency for Toxic Substances and Disease Registry, 2012c).

2.2.2 Health Effects of Cadmium

In recent years, the association between environmental exposure, or low dose exposure, to cadmium and the potential adverse health outcomes have been of particular interest, which builds on early findings among occupationally-exposed cohorts. The literature supports that the kidney is the critical organ for cadmium exposure, with cadmium efficiently retained in the kidney (half-life of 10-30 years in the kidney) and the concentration proportional to that measured in urine (Järup and Åkesson, 2009; Agency for Toxic Substances and Disease Registry, 2012c).

In 1990, Buchet et al. demonstrated that for people who have never been occupationally exposed to cadmium, the low-dose urinary cadmium concentration may be associated with changes in proximal tubular function (Buchet et al., 1990). Additionally, studies across Europe (in Sweden, Poland, Czech Republic, France, and the United Kingdom), the US, and Asia (Japan and China), researchers have found similar results regarding the dose-response relationship between urinary cadmium concentrations and tubular damage (Buchet et al., 1990). This cross-sectional study was a breakthrough study that demonstrated the adverse effects of environmental

cadmium exposure, rather than occupational. Although there was a large sample size, the cross-sectional design makes it difficult to determine when exposure took place and if there is a latency period between exposure and outcome.

In 2006, de Burbure et al. conducted a study to explore the effects of cadmium, lead, mercury, and arsenic among children in three separate European regions, France, Poland, and the Czech Republic (de Burbure et al., 2006). The authors concluded that even at low environmental exposure levels, sometimes with no detectable threshold, heavy metals commonly found in the environment, caused subtle effects on children's renal and dopaminergic systems, with renal hyperfiltration as an early response to lead exposure and subtle tubular effects associated with cadmium exposure, modulated by co-exposure to mercury and lead (de Burbure et al., 2006). While this cross-sectional study provides important insights on low-dose exposure to heavy metals, like cadmium, may have no detectable threshold, repeated biological samples over time would strengthen the findings.

Ferraro et al. analyzed NHANES from 1999-2006 to evaluate the association between cadmium exposure and the risk of chronic kidney disease (Ferraro et al., 2010). Among the 5,426 participants, the average blood cadmium level was 0.40 ± 0.38 $\mu\text{g/L}$ and 0.29 ± 0.35 $\mu\text{g/g}$ for urinary cadmium (Ferraro et al., 2010). After adjusting for the covariates age, gender, race/ethnicity, body mass index and smoking habits, participants with blood cadmium greater than 1 $\mu\text{g/L}$ showed a higher association with both chronic kidney disease (OR 1.48, 95% CI: 1.01, 2.17) and albuminuria (OR 1.41, 95% CI: 1.10, 1.82) based on the multivariable logistic regression analysis (Ferraro et al., 2010). For participants with blood cadmium greater than 1 $\mu\text{g/L}$ and urinary

cadmium greater than 1 µg/g, there was a higher association with albuminuria (OR 1.63, 95% CI: 1.23, 2.16) (Ferraro et al., 2010). In their analysis, the authors' results supported that urinary and blood cadmium concentrations below the accepted thresholds are associated with a higher proportion of kidney disease and albuminuria; however, this study is cross-sectional study (Ferraro et al., 2010).

While renal effects have been well established in the literature, Åkesson et al. reviewed the literature on non-renal health effects of cadmium exposure with respect to human health assessment and found that low exposure, measured as urinary cadmium (mean urinary cadmium levels ranging from 0.05 µg/g creatinine to 13 µg/g creatinine) was associated with effects on bone, including increased risk of osteoporosis and fractures (Åkesson et al., 2014). The various study designs (cross-sectional, retrospective cohort, and prospective cohort), populations (general public and occupational cohorts), countries (Sweden, Belgium, Japan, China, US, and Poland), adjustment or stratification for smoking status, and exposure assessment methods (urine, blood, and/or dietary intake) reduced the likelihood that positive associations found were due to confounding (Åkesson et al., 2014). The associations with bone effects, including a decrease of bone mineral density and increased risk of osteoporotic fractures, seem to occur at low cadmium exposure (as low as 0.5 µg/g creatinine) (Åkesson et al., 2014). These adverse effects on bone appear to occur at lower cadmium exposures than kidney effects (urinary cadmium 0.5-2 versus >4 µg/g creatinine, respectively) (Åkesson et al., 2014). Thus, the authors conclude that the effects on bone from low dose cadmium exposure will likely contribute more than kidney effects to overall risk of adverse effects from cadmium exposure (Åkesson et al., 2014).

In addition to renal effects, cadmium exposure has been associated with adverse cardiovascular outcomes. NHANES data was used to evaluate a prospective association of blood and urine cadmium concentrations with all-cause and cardiovascular mortality in the 1999-2004 US population (Teller-Plaza et al., 2012). The results of this study found that cadmium exposure measured in both blood and urine was prospectively associated with both all-cause and cardiovascular disease mortality, after the adjustment of sociodemographic and cardiovascular disease risk factors, including smoking status (Teller-Plaza et al., 2012). The geometric mean of blood and cadmium was 0.44 µg/L and 0.28 µg creatinine for urinary cadmium (Teller-Plaza et al., 2012). The adjusted hazard ratios for all-cause mortality for blood and urinary cadmium were 1.50 (95% CI: 1.07, 2.10) and 1.52 (95% CI: 1.06, 2.29), respectively (Teller-Plaza et al., 2012). For cardiovascular mortality, the adjusted hazard ratio for blood cadmium was 1.69 (95% CI: 1.03, 2.77) and 1.74 (95% CI: 1.07, 2.83) for urinary cadmium (Teller-Plaza et al., 2012). The adjusted hazard ratio for heart disease mortality for blood cadmium was 1.98 (95% CI: 1.11, 3.54) and 2.53 (95% CI: 1.54, 4.16) for urinary cadmium (Teller-Plaza et al., 2012). Lastly, for coronary heart disease mortality, the adjusted hazard ratio for blood cadmium was 1.73 (95% CI: 0.88, 3.40) and for urinary cadmium, 2.09 (95% CI: 1.06, 4.13) (Teller-Plaza et al., 2012). The authors concluded that cadmium, even at substantially low levels of exposure, remains an important determinant of all-cause and cardiovascular mortality in a representative sample of the US adult population (Teller-Plaza et al., 2012).

Furthermore, a recent study out of Sweden found an association with cadmium exposure and incidence of heart failure (Borné et al., 2015). Laboratory measurements

of blood cadmium concentrations and health questionnaires, which included questions on smoking history and current cardiovascular health status, were administered to participants at baseline and then followed over time, with an average follow-up of 16.8 years (Borné et al., 2015). Overall, the authors found a significantly higher incidence of heart failure in participants in the highest quartile of blood cadmium compared to those in the lowest quartile; however there was no significant association between blood cadmium and incidence of atrial fibrillation (Borné et al., 2015). The median blood cadmium concentration in men was 0.24 µg/L (range: 0.02-5.07 µg/L), while in women, the median blood cadmium concentration was 0.27 µg/L (range: 0.03-4.83 µg/L) (Borné et al., 2015). After adjustment for conventional risk factors and biomarkers (smoking, age, BMI), the hazard ratio for men was 3.91 (95% CI: 1.32, 11.54) and 1.18 (95% CI: 0.49, 2.82) for women (Borné et al., 2015). In sensitivity analysis based on smoking status, for never smokers, the hazard ratio was 2.87 (95% CI: 0.60, 13.85), after adjustment for conventional risk factors and biomarker (Borné et al., 2015). This study adds to the current literature in support of the association of low levels of cadmium in the general population and cardiovascular effects, in addition to the well-established adverse renal effects.

Cadmium is classified as a Group 1 carcinogen (carcinogenic to humans) by the IARC and Class B1 (probable human carcinogen) by US EPA (International Agency on Cancer Research, 2012; United States Environmental Protection Agency, 2014j). The evaluation by IARC is largely based on epidemiological studies of occupational cohorts and animal and toxicological studies that demonstrated an association with occupational exposure to cadmium and lung cancer. Initial studies investigating the potential

association between occupational exposure to cadmium and lung cancer were among US workers (mainly men) who worked in cadmium recovery facilities (Agency for Toxic Substances and Disease Registry, 2012c; International Agency on Cancer Research, 2012). In 1976, Lemen et al. conducted one of the first studies in an occupational cohort that found a significant increase in mortality from malignant neoplasms of the respiratory tract among hourly workers employed for at least two hours between 1940 and 1969 (Agency for Toxic Substances and Disease Registry, 2012c; International Agency on Cancer Research, 2012; Lemen et al, 1976). A second analysis, including longer follow-up through 1978, found significant standardized mortality rates for malignant neoplasms in the respiratory tract (Agency for Toxic Substances and Disease Registry, 2012c; International Agency on Cancer Research, 2012; Thun et al., 1985). This study also categorized workers based on their estimated cumulative cadmium exposure to better understand the dose-response relationship between cadmium and lung cancer (Agency for Toxic Substances and Disease Registry, 2012c; International Agency on Cancer Research, 2012; Thun et al., 1985). For workers in the highest cumulative exposure category (greater than 8-years-mg/m³), there was a 2- to 8-fold increase in the risk of lung cancer mortality (Agency for Toxic Substances and Disease Registry, 2012c; International Agency on Cancer Research, 2012; Thun et al., 1985). As the relationship between occupational cadmium exposure and lung cancer mortality was further studied, subsequent studies were able to address confounding issues (for example, accounting for smoking status) improve the quality of studies, which in turn further supported that there is an association between occupational cadmium exposure

and cancer, particularly lung, mortality (Agency for Toxic Substances and Disease Registry, 2012c; International Agency on Cancer Research, 2012).

However, recent literature on environmental cadmium exposure in the general population has demonstrated that low dose environmental exposure to cadmium may be associated with cancer. In order to evaluate the association between the creatinine-corrected urinary cadmium levels with all-cause and cause-specific mortality in the general US population, Menke et al. analyzed the data from 13,958 participants in the Third NHANES (1988-1994) and followed them through December 31, 2000 for mortality (Menke et al., 2009). Overall, the geometric mean levels of urinary cadmium per gram of urinary creatinine was 0.28 µg/g for men and 0.40 µg/g for women (Menke et al., 2009). The hazard ratios for men in the adjusted multivariable model, which included smoking status, were 1.28 (95% CI: 1.15, 1.43) for all-cause mortality, 1.55 (95% CI: 1.21, 1.98) for cancer mortality, 1.21 (95% CI: 1.07, 1.36) for cardiovascular disease mortality, and 1.36 (95% CI: 1.11, 1.66) for coronary heart disease mortality (Menke et al., 2009). For women, the hazard ratios in the adjusted multivariable model were 1.06 (95% CI: 0.96, 1.16), 1.07 (95% CI: 0.85, 1.35), 0.93 (95% CI: 0.84, 1.04), and 0.82 (95% CI: 0.76-0.89) for all-cause, cancer, cardiovascular disease, and coronary heart disease mortality, respectively (Menke et al., 2009). Although low dose, environmental cadmium exposure was associated with an increased risk of all-cause, cancer, and cardiovascular disease mortality among men, but not among women, the potential public health implications of these findings are substantial because there is widespread exposure to low levels of cadmium in the general population (Menke et al., 2009).

Adams et al. also examined the Third NHANES cohort to examine the relationship between cadmium exposure and cancer mortality, but unlike Menke et al., Adams et al. investigated the relationship to specific cancers (Adams et al., 2012). There were associations between creatinine-corrected urinary cadmium samples and cancer mortality in both men and women, although the evidence of associations in women was weaker than in men (Adams et al., 2012). The geometric mean for urinary cadmium in men was 0.525 µg/g (95% CI: 0.235, 0.271) and 0.352 µg/g (95% CI: 0.327, 0.379) in women (Adams et al., 2012). Among men in the upper quartile of urinary cadmium, the adjusted hazard ratio for non-Hodgkin lymphoma was 25.83 (95% CI: 3.93, 169.6), 7.25 (95% CI: 1.77, 29.80) for pancreatic cancer, 3.22 (95% CI: 1.26, 8.25) for lung cancer, and 1.86 (95% CI: 0.31, 11.13) for leukemia (Adams et al., 2012). For women in the upper quartile of urinary cadmium, the adjusted hazard ratio for ovarian cancer was 2.40 (95% CI: 0.66, 8.69), 1.82 (95% CI: 0.99, 3.33) for lung cancer, 1.67 (95% CI: 0.55, 5.07) for leukemia, and 1.24 (95% CI: 0.58, 2.63) for pancreatic cancer (Adams et al., 2012). In men, much of excess risk was associated with lung cancer mortality, in both smokers and non-smokers, which is consistent with the results from a Belgian population-based prospective study (Adams et al., 2012). The authors conclude that cadmium exposure, independent of cigarette smoking, was most significantly associated with lung cancer, non-Hodgkin lymphoma, and pancreatic cancer mortality in men, while in women, the associations of urinary cadmium with mortality from specific cancers were less clear (Adams et al., 2012).

Julin et al. examined a cohort of Swedish men to assess the relationship between dietary cadmium exposure and prostate cancer incidence and mortality, based

on food frequency questionnaires and a national cancer registry (Julin et al., 2012). Dietary cadmium exposure was associated with an increased risk of total prostate cancer tumors (Julin et al., 2012). The increased risk for prostate cancer was more pronounced for localized prostate tumors for lean men who reported that they were smokers (Julin et al., 2012). The mean estimated energy-adjusted cadmium exposure among the 41,089 men at baseline was 19 ± 3.7 μg per day (Julin et al., 2012). In the adjusted multivariable model, dietary cadmium exposure was positively associated with overall prostate cancer incidence, with a relative risk of 1.13 (95% CI: 1.03, 1.24) (Julin et al., 2012). For localized cases of prostate cancer, the relative risk was 1.29 (95% CI: 1.01, 1.26), 1.05 (95% CI: 0.87, 1.25) for advanced cases, and 1.14 (95% CI: 0.86, 1.51) for fatal cases (Julin et al., 2012).

Lastly, in a case-control study by Luckett et al., after a comprehensive assessment of potential sources of exposure, including smoking status, dietary sources like drinking water, and occupational sources, they found an increased risk of pancreatic cancer with increased urinary cadmium concentrations (Luckett et al., 2012). The levels of urinary cadmium were categorized into incremental quartiles of 0.5 $\mu\text{g/g}$ creatinine and the top quartile incorporating any levels greater than 1.5 $\mu\text{g/g}$ creatinine (Luckett et al., 2012). Using the lowest urinary cadmium concentration category of less than 0.5 $\mu\text{g/g}$ creatinine as the referent, the odds ratios for the subsequent second, third, and fourth categories were 3.34 (95% CI: 1.38, 8.07), 5.58 (95% CI: 2.03, 15.34), and 7.70 (95% CI: 3.06, 19.34), respectively (Luckett et al., 2012). In addition to monotonic increased risk for pancreatic cancer based on urinary cadmium concentrations, the authors also found increased risk of pancreatic cancer for those with an occupation as a

plumber, pipefitter, or welder (odd ratio of 5.88, 95% CI: 1.33, 26.01), high consumption of red meat (odds ratio of 6.18, 95% CI: 2.28, 16.76), and grains (odds ratio: 3.38, 95% CI: 1.10, 10.36) (Luckett et al., 2012). For current smokers, the odds ratio was 1.52 (95% CI: 0.59, 3.94) and for smokers that had greater than 80 pack-years, the odds ratio was 2.82 (95% CI: 0.82, 9.66) (Luckett et al., 2012). The findings of this study indicate that cadmium may be a potential pancreatic carcinogen and that both environmental and occupational exposures together should be considered in future studies (Luckett et al., 2012).

Although the initial classification of cadmium as carcinogenic to humans by IARC was largely based on epidemiological studies of occupational cohorts, current literature on the impacts of cadmium through low dose environmental exposures, suggests an association with dietary and environmental exposures to cadmium and cancer (Åkesson et al., 2014; Hartwig, 2013).

In summary, five cross-sectional studies (four which analyzed NHANES data), three prospective cohort studies, one case-control study, and one review found associations between cadmium exposure and adverse health outcomes. Of these studies, four studies found positive associations between exposure to cadmium and renal outcomes, including tubular effects and kidney disease (Buchet et al., 1990; de Burbure et al., 2006; Ferraro et al., 2010; Åkesson et al., 2014). In addition to kidney effects, Åkesson et al. found low concentrations of urinary cadmium were associated with effects on bone, including increased risk of osteoporosis and fractures, and that these effects on bone appear to occur at lower cadmium exposures than kidney effects (Åkesson et al., 2014). Although kidneys are the target organs for exposure to

cadmium, three studies found positive associations with cardiovascular outcomes, including cardiovascular mortality (Menke et al., 2009; Teller-Plaza et al., 2012; Borné et al., 2015). Furthermore, four studies found associations between low cadmium exposures (dietary and environmental) were associated with cancers - lung cancer, non-Hodgkin lymphoma, and pancreatic cancer mortality - in men; however, in women, the associations between cadmium and cancers were less clear (Menke et al., 2009; Adams et al., 2012; Julin et al., 2012; Lockett et al., 2012). These few studies demonstrate that cadmium exposure, even at environmental low-doses, are associated with adverse health outcomes, including mortality. Because of the numerous studies that have demonstrated adverse renal effects from chronic exposure to cadmium, the MCL for cadmium is 5 ppb (0.005 ppm) (United States Environmental Protection Agency, 2014b).

2.3 Lead

Lead, like other heavy metals, is a naturally occurring metal in the environment that is readily accessible and widely distributed in ore deposits (Agency for Toxic Substances and Disease Registry, 2007b). Because of its chemical and physical properties of corrosion resistance, density and low melting point, lead has been widely used in pipes, solder, weights, storage batteries, paint, and gasoline (Agency for Toxic Substances and Disease Registry, 2007b; United States Environmental Protection Agency, 2015b). Lead can enter the environment from historical and current uses. Leaded gasoline was a major source of exposure to lead to the general population between the 1920s and 1970s, before the use of lead in gasoline was banned (Agency

for Toxic Substances and Disease Registry, 2007b; United States Environmental Protection Agency, 2015b). Lead-based paint, smelters, refineries, and mining practices are the major sources of lead exposure to the general population (Agency for Toxic Substances and Disease Registry, 2007b; United States Environmental Protection Agency, 2015b). From these processes, lead is emitted into the air and can settle in water and soil/sediment (Agency for Toxic Substances and Disease Registry, 2007b; United States Environmental Protection Agency, 2015b). The solubility of lead and its compounds in water is a function of pH, hardness, salinity, and the presence of humic material. Lead has the highest solubility in soft, acidic water (Agency for Toxic Substances and Disease Registry, 2007b). In the US, lead in surface waters and groundwater ranges from 5 to 30 ppb, with more than 99% of all publicly supplied drinking water containing 0.005 ppm (Agency for Toxic Substances and Disease Registry, 2007b). In soil, nature levels of lead range from 50 to 4,000 ppm, but in areas where there is heavy mining, smelting, and/or refining activities, the amount of lead in the soil is substantially higher (Agency for Toxic Substances and Disease Registry, 2007b; United States Environmental Protection Agency, 2015b). Based on 2011-2012 survey years, the reported geometric mean for blood lead levels was 0.973 microgram per deciliter ($\mu\text{g}/\text{dL}$) (95% CI: 0.916, 1.04), for a sample size of 7,920 (Centers for Disease Control and Prevention, 2015a). The geometric mean for urinary lead, creatinine corrected was 0.409 $\mu\text{g}/\text{g}$ creatinine (95% CI: 0.380, 0.440) with a sample size of 2,502, based on data from survey years 2011-2012 (Centers for Disease Control and Prevention, 2015a).

2.3.1 Routes of Exposure for Lead

The use of lead and lead-based solder in water service pipes in residences and distribution systems can cause lead to be leached into drinking water (Agency for Toxic Substances and Disease Registry, 2007b). The main exposure pathway of lead to the general population is through ingestion of contaminated water (Agency for Toxic Substances and Disease Registry, 2007b). Studies have found that plumbing components, water quality parameters, water treatment and the presence of other contaminants and metals may influence the amount of lead that is present in drinking water (Guidotti et al., 2007; Pieper et al., 2015; Knowles et al., 2015; Masters et al., 2015). Homes built prior to the 1980s are likely to have lead solder connecting copper pipes, resulting in an estimated 10 million American homes and buildings that receive water from service lines that are at least partially containing lead (Plumbing Manufacturers International, n.d.; Delaney, 2016).

Additionally, because lead-based paint was largely used in homes and buildings built prior to 1978, especially before 1940, inhalation of contaminated lead dust and ingestion of lead paint chips, especially by children, can also be a leading source of lead exposure to the general population (Agency for Toxic Substances and Disease Registry, 2007b; United States Environmental Protection Agency, 2015b).

Workers in industries like lead smelting and refining, soldering, steel welding, battery manufacturing, construction and demolition, and municipal waste incinerators, have higher exposures to lead (Agency for Toxic Substances and Disease Registry, 2007b). The main route of exposure to occupational lead is through inhalation, with accidental ingestion a secondary route (Agency for Toxic Substances and Disease

Registry, 2007b). In lead smelting and refineries, the mean concentrations of lead in air can reach 4,470 $\mu\text{g}/\text{m}^3$ and for welders, the average lead concentrations in air can reach 1,200 $\mu\text{g}/\text{m}^3$ (Agency for Toxic Substances and Disease Registry, 2007b).

The amount of lead absorption in the gastrointestinal tract is between 3-10% in adults, and 40-50% in children, with absorption rates decreased by the presence of food (Agency for Toxic Substances and Disease Registry, 2007b). Once lead is absorbed through the GI tract, lead is distributed and stored, with 94% contained in bones in adults and 73% in teeth in children (Agency for Toxic Substances and Disease Registry, 2007b). The amount of lead absorbed through inhalation is dependent on particle size, solubility, and the pattern of regional distribution within the respiratory tract (Agency for Toxic Substances and Disease Registry, 2007b). Once lead is in the lungs, approximately 95% is absorbed (Agency for Toxic Substances and Disease Registry, 2007b). In blood, lead has a half-life of approximately 30 days and in bone, the half-life is 27 years (Agency for Toxic Substances and Disease Registry, 2007b).

2.3.2 Health Effects of Lead

Exposure to lead has been extensively described in the public and environmental health literature. Regardless of the route of entry into the body (ingestion or inhalation), the nervous system is the target organ of lead, for both adults and children (Agency for Toxic Substances and Disease Registry, 2007b). The first studies investigating the adverse effects of lead were among occupational cohorts, which initially focused on cerebrovascular disease and mortality (Agency for Toxic Substances and Disease Registry, 2007b). As with adults in occupational cohorts, high exposure to lead caused

adverse effects in children, mainly encephalopathy (Agency for Toxic Substances and Disease Registry, 2007b). Several studies out of Baltimore, MD were among the first to find an association with blood lead levels in children and acute encephalopathy, based on data from 1930 to 1970 (Agency for Toxic Substances and Disease Registry, 2007b). Another early study was published in 1943 and detailed the cognitive effects of childhood lead exposure, especially the long term impacts of lead poisoning on mental development (Byers and Lord, 1943).

As research progressed through the 1970s, the neurobehavioral effects in children from exposure to lead were becoming more prominent in the literature, and the focus of lead exposure shifted from high-dose exposure to low-dose exposure, especially as leaded gasoline and lead-based paint were banned (Agency for Toxic Substances and Disease Registry, 2007b). With growing knowledge surrounding this issue, the 1970s NHANES started collecting and compiling blood lead levels (Centers for Disease Prevention and Control, 2012a). In 1978, an estimated 13.5 million children (1-5 years of age) had blood lead levels greater than or equal to 10 µg/dL, based on NHANES data (Centers for Disease Control and Prevention, 2012a).

Through the 1980s and 1990s, there was a plethora of epidemiological studies that explored the relationship between exposure to lead and neurodevelopmental outcomes, especially in children. Bellinger et al. collected blood samples at birth, six, 12, 18, 24, and 57 months of age and performed cognitive testing with the McCarthy Scales of Children's Abilities at 57 months in a cohort of 170 children in the Boston area (Bellinger et al., 1991). The average blood lead level for children in this cohort at 24 months of age was 6.8 ± 6.3 µg/dL (Bellinger et al., 1991). For those children that had

umbilical cord blood lead levels greater than 10 µg/dL, after adjustment for confounding prenatal elevated blood lead was not associated with cognitive development at 57 months of age (Bellinger et al., 1991). However, for children who had postnatal blood lead levels greater than 10 µg/dL, especially at 24 months of age, had poorer performance on cognitive testing at 57 months of age (Bellinger et al., 1991).

Baghurst et al. conducted one of the first longitudinal cohort studies that evaluated environmental exposure to lead and the intelligence of children through seven years of age in the Port Pirie cohort (Baghurst et al., 1992). Measuring IQ through the Wechsler Intelligence Scale for Children (WISC-R) in 494 children at seven years of age, the authors found an inverse relationship between IQ and both antenatal and postnatal blood lead concentrations (Baghurst et al., 1992). In a multiple regression model, the relationship with exposure to lead is still evident in postnatal blood samples, especially in 15 months to four years of age, after adjustment for a multitude of covariates, including sex, parents' level of education, maternal age at delivery, parents' smoking status, socioeconomic status, quality of home environment, maternal IQ, birth weight, birth order, feeding method, duration of breast feeding, and whether the child's natural parents were living together (Baghurst et al., 1992). The authors reported when the average blood lead level increased from 10 µg/dL to 30 µg/dL at 15 months and two, three, and four years of age, the estimated reduction in IQ ranged from four to five percent, of a reduction of 4.4 (95% CI: 2.2, 6.6) points to 5.3 (95% CI: 2.8, 7.8) points (Baghurst et al., 1992). The results of this study showed that in what was considered low-level exposure to lead (mean concentration of 6.2 µg/dL of lead in antenatal

samples), there was an inverse association with neuropsychological development through the age seven (Baghurst et al., 1992).

Wasserman et al. conducted a prospective cohort study to assess the association of lead exposure and early development, in which pregnant women were recruited from a lead smelting town and an exposed town in Yugoslavia and their children were followed through seven years of age (Wasserman et al., 1997). Lifetime lead exposure was estimated by the area under the blood lead versus time curve, with intelligence measured by the Wechsler Intelligence Scales for Children (WISC-III) (Wasserman et al., 1997). Complete data on blood lead levels, intelligence, and relevant sociodemographic covariates were available for 261 children (Wasserman et al., 1997). In a regression model, adjusted for sociodemographics, there was a statistically significant adverse impact of blood lead levels on school age Full Scale, Verbal, and Performance IQ (Wasserman et al., 1997). A change in lifetime blood lead levels from 10 $\mu\text{g/dL}$ to 30 $\mu\text{g/dL}$ was related to an estimated decrease of 4.3 points on Full Scale IQ, 3.4 points on Verbal IQ, and 4.5 points on Performance IQ (Wasserman et al., 1997). The authors state that the findings of their study offers support that perceptual-motor skills are significantly more sensitive to lead exposure than are the language-related aspects of intelligence (Wasserman et al., 1997).

In 2003, Canfield et al. published their findings that even at blood lead concentrations below 10 $\mu\text{g/dL}$, there were intellectual impairments in children (Canfield et al., 2003). At the time of the publication, 10 $\mu\text{g/dL}$ was the level of concern set by CDC. This article measured blood lead in 172 children at 6, 12, 18, 24, 36, 48, and 60 months of age, who were previously enrolled into a dust-control efficacy study, and also

administered the Stanford-Binet Intelligence Scale at 36 and 60 months of age (Canfield et al., 2003). The authors found that at intellectual functioning at 36 and 60 months of age was inversely associated with blood lead levels, even when the peak level was below the CDC and World Health Organization (WHO) level of concern of 10 $\mu\text{g}/\text{dL}$ (Canfield et al., 2003). This finding was consistent in unadjusted and adjusted models for lifetime average, concurrent, and peak lead levels (Canfield et al., 2003). In linear mixed models, the authors found the estimated IQ loss to be 4.6 points for each increase of 10 $\mu\text{g}/\text{dL}$ of blood lead; however, the estimated IQ loss was considerably greater for children whose blood lead levels remained below 10 $\mu\text{g}/\text{dL}$ (Canfield et al., 2003). For the nonlinear mixed model, there was an estimated decline in IQ of 7.4 points as the lifetime average blood lead level increased from 1 to 10 $\mu\text{g}/\text{dL}$ (Canfield et al., 2003). The results in this study suggested that there are adverse neurological effects in children, even when their blood lead level is below 10 $\mu\text{g}/\text{dL}$, the CDC's level of concern (Canfield et al., 2003).

Furthermore, consistently through the literature, there are a multitude of studies that have demonstrated the adverse outcomes associated with lead exposure in infants and children and the implications on long-term effects, specifically neurodevelopmental effects. The current literature suggests that there is no threshold for the adverse effects of lead in children (Agency for Toxic Substances and Disease Registry, 2007b). Despite this consensus and the known adverse effects of lead, especially in children, exposure to lead remains a widespread public health issue today. A recent study showed that in Chicago, early childhood lead exposure is associated with poorer achievement on standardized reading and math tests in third grade students enrolled at

Chicago Public Schools, even at very low blood lead levels (Evens et al., 2015). Among 47,168 students, the mean blood lead level was 4.81 ± 2.22 $\mu\text{g/dL}$ (Even et al., 2015). However, a total of 10,182 students had blood lead levels above 10 $\mu\text{g/dL}$, which was 17.8% of the full sample of 57,350 students. The mean blood lead levels are based on children who had blood lead tests reported to the Chicago Department of Public Health between 1996 and 2006 (Evens et al., 2015). After controlling for gender, race/ethnicity, poverty, maternal education, very low birth weight, and early preterm birth, children who had higher blood lead levels scored significantly lower in reading and math, and had significantly higher failure rates (Evens et al., 2015). Even at blood lead levels below the recommended level of 5 $\mu\text{g/dL}$, early childhood exposure to lead has a negative impact on school performance (Evens et al., 2015).

In another study in Montreal, researchers evaluated lead exposure from water sources in children, unlike the focus of most studies, which solely focus on lead-based paint as the exposure (Ngueta et al., 2015). Although it has been well established that lead and its compounds are ubiquitous in drinking water supplies, few studies have truly examined the role of lead in water on blood lead levels (Ngueta et al., 2015). The authors estimated the dose-response relationship between drinking water cumulative lead exposure and blood lead in children 1 to 5 years old (Ngueta et al., 2015). Of the 298 study participants and their families, the geometric mean blood lead level was 1.3 $\mu\text{g/dL}$ (95% CI: 0.5, 3.6), similar to previous studies in US children (geometric mean 1.3 $\mu\text{g/dL}$ (95% CI: 1.3, 1.4)) (Ngueta et al., 2015). The authors developed a model to estimate the cumulative water lead exposure index, which accounted for environmental samples of water, dust and paint, child's characteristics and habits, the parents'

characteristics and lifestyle habits, child's nutrition (daily water consumption patterns, frequency of meals), the total number residing in each household, and additional factors such as distance to roadways, nearby industries, water temperature, and season (Ngueta et al., 2015). Using this cumulative lead exposure index through drinking water (CWLEI), the authors found a positive association with blood lead levels and a clear dose-response pattern starting at 0.7 μg lead per kg of body weight (Ngueta et al., 2015). For every one unit of increase in the CWLEI, there would be a resulting 10.5% increase in blood lead levels (Ngueta et al., 2015). Furthermore, based on the average water consumption of the cohort (approximately 21 mL/kg/day), the CWLEI model suggests that an increase of 1 ppb in water lead concentration would result in a 35% increase in the blood lead level after 150 days (5 months) of exposure (Ngueta et al., 2015). Because of the low clearance of blood lead, estimating cumulative exposure to lead should substantially improve the ability to accurately estimate the effects of lead exposure resulting from water (Ngueta et al., 2015). The CWLEI model created by Ngueta et al. demonstrated how water lead concentrations tested below regulatory guidelines, can still impact the blood lead levels in children after long term exposure (Ngueta et al., 2015). This study suggests that water lead levels in addition to lead paint exposure should be accounted for in future studies that estimate blood lead levels in children based on lead exposures.

One recent study by Rodrigues et al. investigated the associations of lead, arsenic and manganese and neurodevelopmental outcomes at 20 to 40 months of age in Bangladeshi children and the possible interactions from lead, arsenic and manganese on these outcomes from exposure to contaminated drinking water (Rodrigues et al.,

2016). Data was available on drinking water source during pregnancy and at ages 1, 12, and 20-40 months of age, as well as blood lead levels at 20-40 months of age, and neurodevelopmental outcomes assessed by the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III) for 524 children participating in an ongoing prospective birth cohort study (Rodrigues et al., 2016). The results from this study were consistent with the literature in that with higher blood lead levels, there was a decrease in cognitive scores (Rodrigues et al., 2016). The results from this study provides support for the need of future research on the potential implications of how exposure to multiple heavy metals in drinking water may be associated with neurodevelopmental effects in children. As previously mentioned, exposure to lead (and other heavy metals) in drinking water may play a larger role in neurodevelopmental outcomes in children.

Numerous studies assessing exposure to lead found cognitive and neurodevelopmental effects in children, even at blood lead levels below 10 µg/dL (Bellinger et al., 1991; Baghurst et al., 1992; Wasserman et al., 1997; Canfield et al., 2003; Evens et al., 2015). These cognitive and neurodevelopmental effects have a lasting impact have created federal regulations to reduce and eliminate lead exposure. Although many of the studies detailed above focus on exposure to paint contaminated with lead, other low dose and environmental exposure cannot be ruled out as potentially sources that may result in adverse effects (Ngueta et al., 2015). Recent events in Flint, MI have indicated that lead in drinking water should be considered and evaluated as plausible source that contribute to blood lead levels in children (Bellinger, 2015; Sanburn, 2016; Hanna-Attisha, et al., 2016; Milman and Glenza, 2016). Because of the

adverse effects lead can cause, the US EPA TT action level for lead is 15 ppb and 1300 ppb for copper (United States Environmental Protection Agency, 2014d).

2.4 Population-Based Heavy Metal Exposure

Population-based estimates for heavy metal exposure are limited to national health survey data. In the US, two surveillance datasets are used to provide information on general population exposure levels with minimal information on adverse health outcomes. These two dataset are NHANES and NPDS, which provide information on general population exposure levels, but have minimal information about adverse health outcomes.

NHANES is a nationally representative sample with persons located in counties across the country and include includes demographic, socioeconomic, dietary, and health-related questions (Centers for Disease Control and Prevention, 2014a). Data from NHANES can be used to determine the prevalence of major disease and risk factors for diseases, as well as exposures to contaminants, including heavy metals (Centers for Disease Control and Prevention, 2014a). The measured concentrations for heavy metals in biological specimens collected by NHANES can be seen in Table IV.

TABLE IV
SUMMARY OF BIOLOGICAL MEASURES OF HEAVY METALS FROM NHANES

Metal	Urinary	Blood	Survey Year
Antimony	0.06 (0.06, 0.06) µg/g creatinine	-	2009-2010
Arsenic, total	7.77 (6.85, 8.81) µg/g creatinine	-	2011-2012
Cadmium	0.18 (0.17, 0.19) µg/g creatinine	0.28 (0.27, 0.29) µg/L	2011-2012
Lead	0.41 (0.38, 0.44) µg/g creatinine	0.97 (0.92, 1.04) µg/dL	2011-2012
Manganese	0.14 (0.13, 0.16) µg/g creatinine	9.35 (9.19, 9.51) µg/L	2011-2012
Mercury, total	0.36 (0.33, 0.41) µg/g creatinine	0.70 (0.62, 0.80) µg/L	2011-2012

Another way to assess the impact of heavy metal exposure is to look at cases of poisonings from heavy metals. Poisonings from heavy metals may be the result of acute exposure – environmental, accidental, intentional, or even occupational – or chronic exposure, such as occupational exposures. The American Association of Poison Control Centers (AAPCC) compiles and reports an annual number of calls and cases of heavy metal poisonings from 55 poison centers in the US to the National Poison Data System (NPDS) (Mowry et al, 2014). In 2013, the AAPCC recorded the following number of single exposures to heavy metals: 644 arsenic, 36 cadmium, 175 (trivalent) chromium, 2076 lead, 34 manganese, 112 mercury, 1174 elemental mercury, 1850 other, and 63 unknown (Mowry et al, 2014). Between the biological measures from NHANES and the annual number of heavy metal poisonings by AAPCC, there is evidence exposure to heavy metals is widespread in the general US population and remains a public health issue.

2.5 Methodologies for Detecting Heavy Metals in Drinking Water

2.5.1 Laboratory-based Detection Methods

There are several laboratory-based methods for detecting heavy metals in drinking water. ICP-MS is extremely sensitive, with detection limits between 1,000 and 100,000 ppt (American Public Health Association et al., 2012). The sample is placed into the ICP-MS instrument and introduced into an argon-based, high temperature radio-frequency plasma, usually via pneumatic nebulization (American Public Health Association et al., 2012). Energy transfers from the plasma to the sample stream, where the target element (in this case the metals) dissolve, become atomized and then

ionized (American Public Health Association et al., 2012). The resulting ions are then extracted from the plasma through a differential vacuum interface and then separated based on the mass-to-charge ratio by a mass spectrometer (American Public Health Association et al., 2012).

ICP-AES is less sensitive than ICP-MS, but is commonly used for detecting heavy metals in drinking water (Lajunen and Peramaki, 2004). Briefly, the sample is placed into the instrument, where a plasma source, which is both an atomization and excitation source, is introduced, resulting in the sample being evaporated and dissociated into free atoms and ions (Lajunen and Peramaki, 2004). Energy is supplied causing the free atoms and ions to excite to higher energy states (Lajunen and Peramaki, 2004). In this excited state, atoms and ions are unstable, and lose energy through collisions with other particles or transition to a lower energy level where radiation is emitted (Lajunen and Peramaki, 2004). This radiation is measured through the emitted wavelengths (Lajunen and Peramaki, 2004).

2.5.2 Field-based Detection Methods

There is widespread interest in developing field-based methods for detection of heavy metals in water. Previous studies have considered a number of different types of field-based methods, but most were designed to detect naturally occurring arsenic in drinking water globally (Rahman et al., 2002; Van Geen et al., 2005; Steinmaus et al., 2006; Jakariya et al., 2007; Baghel et al., 2007; Sankararamakrishnan et al., 2008). A number of various water quality parameters and sampling factors – incubation time, temperature, preservative, bottle type – can impact the arsenic concentrations

determined by these field-based methods (Van Geen et al., 2005). The majority of current field-based methods use color chart matching which only provides an estimated concentration or concentration range and depends on personnel to subjectively identify the coloration. Although biosensors for heavy metals have been developed and successfully tested in controlled laboratory settings (Raja and Selvam, 2011; Rategarpanah et al., 2013; Shing et al., 2008; Cerminati et al., 2015; Chu et al., 2015), they have not been widely tested in field conditions nor are they commercially available. Furthermore, commercially marketed field-based methods, or kits, available for testing for heavy metals in drinking water are only able to test a single heavy metal, mainly arsenic, and testing for other heavy metals such as cadmium and lead is not feasible.

2.5.2.1 Biosensor Detection Methods

Biosensors offer an alternative field-based method; however, there are limited studies to date that explore the use of field-based biosensor methodology for detection of heavy metals such as cadmium and lead in drinking water. Biosensors could be used in real-time for direct quantification, or for initial screening that would allow for laboratory-based analysis of fewer samples.

A biosensor is an analytical device that consists of immobilized biological material in intimate contact with a compatible transducer, which will convert the biochemical signal into a quantifiable electrical signal (Rategarpanah et al., 2013). Biosensors may rely on analysis of gene expression, utilizing a promoter of interest and a reporter gene. In this way, the gene expression would serve as a measure of the availability of a specific pollutant in the environment (Raja and Selvam, 2011;

Rategarpanah et al., 2013; Shing et al., 2008; Tao et al., 2013). Data on the use of biosensor methodologies to detect heavy metals is still scarce. Several researchers have been successful in developing different biosensors, fewer have explored the reliability and accuracy of these biosensors in the field (Raja and Selvam, 2011; Rategarpanah et al., 2013; Shing et al., 2008; Tao et al., 2013; Gu et al., 2004; Bentley et al., 2001; Horton et al., 1989; Shetty et al., 2003; Tauriainen et al., 1998; Guo et al., 2015; Li et al., 2014; Sochor et al., 2011; Shetty et al., 2003). There is a need to test the performance of the biosensor technology in the field setting, where conditions may vary considerably from the controlled setting of a laboratory.

In 2013, US EPA conducted testing for the detection of lead in water using a newly developed field-based biosensor system manufactured by ANDalyze. The system consists of a handheld fluorimeter and a consumable metal-specific test kit. The fluorescent and colorimetric sensor technology uses *in vitro* selection, a combinational biology method, to obtain catalytic DNA with high specificity and selectivity for metal ions (Lu, 2009). The DNA is labeled with a fluorophore/quencher pair and in the presence of metal ions, the catalytic DNA is cleaved, resulting in a dramatic increase of fluorescence signal; the greater the concentration of the metal, the greater the fluorescence (Lu, 2009). The metal-specific biosensors are reported to be highly selective and highly sensitive, with a limit of detection as low as 11 ppt (Lu, 2009). For lead, the detection range of the biosensor system is 2 to 100 ppb (ANDalyze, 2015).

Four performance tests of the ANDalyze for lead laboratory and environmental water samples was performed by US EPA (Yates et al, 2013). First, the determination

of the limit of detection (DLOD) was determined to be 1.534 ppb for lead, with coefficient of variance (CV) ranging from 0.03 to 0.13 (Yates et al, 2013).

The second test that was conducted evaluated the accuracy and precision of the biosensor system for finished drinking water samples. The calculated CV was the reported accuracy measure. The samples were obtained from a water fountain, bottled water, and finished drinking water collected from the effluent of a local water treatment facility (Yates et al, 2013). These samples were analyzed in triplicate, with one set of samples of each type not spiked and a second set spiked with 25 ppb of lead (Yates et al, 2013). The CVs for water fountain, bottled water, and finished drinking water were 0.14, 0.03, and 0.10, respectively (Yates et al, 2013).

The third test was focused on the accuracy and precision of the ANDalyze system in recovering lead from four different environmental spiked water samples; freshwater from a river, freshwater from a reservoir, raw groundwater collected at the source of a drinking water treatment facility, and seawater (Yates et al, 2013). Again, samples were analyzed in triplicate, with one set of samples not spiked and the second set spiked with 25 ppb of lead (Yates et al, 2013). The CVs for river water and reservoir water were similar, 0.07 and 0.08, respectively, with the CV for raw well water at 0.19 (Yates et al, 2013). The CVs for seawater were significantly higher than from other environmental water samples, 0.42 and 0.32, but this was to be expected due to the high salinity of the samples (Yates et al, 2013).

Lastly, the accuracy and precision of the biosensor system in recovering lead spikes in wastewater effluent samples were tested (Yates et al, 2013). Three types of wastewater samples were analyzed: two separate effluent samples from two traditional

activated sludge treatment facilities treating domestic wastewater and one sample from effluent from a metal finishing works. The CVs for the three wastewater effluent samples varied, with the CV of 0.05 from wastewater facility 1, a CV of 0.10 from wastewater facility 2, and a CV of 0.5 from the effluent of the metal finishing works (Yates et al, 2013). Overall, the average percent recoveries ranged from 74% (initial demonstration capability) to 168% (raw well water).

US EPA concluded that the detection of lead by the ANDalyze system was subject to water quality characteristics dictated by environmental conditions and water quality characteristics artificially imparted on synthetic environmental or laboratory samples (Yates et al, 2013). However, there was a major limitation with the ETV report: if on-site calibration failed after the second attempt or if acceptance criteria (quality control) was not met, samples were not analyzed, and the number of samples that did not meet this criteria was not reported (Yates et al, 2013). The proportion of samples that was not able to be analyzed is an important measure of performance, because it indicates how frequently information would not be obtained. The calculated accuracy and precision measures in this report may not be an accurate measure, as they are reflecting the total number of samples that met acceptance criteria rather than the total of samples attempted to be sampled, and thus may over-estimate the performance of the method.

2.6 Detection Methods for Heavy Metal Mixtures

With the multitude of contaminants in the environment, from both naturally occurring and anthropogenic sources, these contaminants are frequently encountered

as mixtures, and the behavior in a mixture may not correspond to that predicted from data on the pure compounds (Altenburger et al., 2003). Concurrent exposure to lead or cadmium may produce additive or synergistic interactions or even new effects that are not seen in single component exposure (Wang and Fowler, 2008). The simultaneous presence of two or more substances may alter physicochemical properties of components such as solubility and this in turn may affect bioavailability of a sample (Altenburger et al., 2003). The effectiveness of screening tools and quantification of detection limits are challenged by chemical mixtures, resulting in less accurate results (Brack et al., 2015; Altenburger et al., 2015).

Few studies have addressed the accuracy of metal quantification or detection when present in a mixture (Brack et al., 2015; Altenburger et al., 2015). Yildirim et al. evaluated the timing, sensitivity, specificity, resistance to background interference and reusability of a DNAzyme-based lead biosensor in tap water and from tertiary effluent from two wastewater treatment plants (Yildirim et al., 2014). In the presence of mercury, calcium, copper, iron, manganese, nickel, magnesium, cobalt, and silver, the sensor did not exhibit significant response (greater than 20% compared to a lead control) to other metal ions (Yildirim et al., 2014). The authors state that the high specificity for lead was due to the specificity of the DNAzyme for lead ions (Yildirim et al., 2014). The results of this study demonstrated that a DNA-based sensor can be metal-specific, in this case lead specific, and that the biosensor was precise, accurate, with good recovery, even when tested with other metal interferences.

Li et al. tested the performance of a paper-based sensor in detecting and reporting heavy metals in water, which could print the chemical symbols of the

corresponding heavy metal with the specific indicator systems in a format of the periodic table of elements (Li et al., 2015). The paper sensor would be dipped into a water sample, and the sensor would reveal the testing results if the concentrations of the heavy metal were higher than legislated standards (Li et al., 2015). The authors examined the interference tolerance of the paper sensor for each metal ion, with and without the presence of interfering ions of copper, chromium, nickel, iron, and zinc (Li et al., 2015). In the presence of interfering ions, there was no significant interference observed (Li et al., 2015). Additionally, the paper sensor showed a mean error within 10% of the results of a gold standard, ICP-AES (Li et al., 2015).

Cerminati et al. conducted a laboratory-based study evaluating the design, construction, and calibration of a whole-cell fluorescence biosensor device that simultaneously reports the presence of mercury, lead, cadmium, and/or gold ions in water by using a MerR family protein as the sensor/regulatory protein (Cerminati et al., 2015). This study also assessed the biosensor's response to samples spiked with multiple heavy metal mixtures (Cerminati et al., 2015). The authors found that there was no interference between the metal observed (mercury, lead, cadmium, and/or gold) when in complex mixtures (Cerminati et al., 2015). The induction coefficient and fluorescence of the biosensor were greatest when in a combined heavy metal mixture sample of four inducer metals (Cerminati et al., 2015). However, this biosensor was not designed and constructed to be metal-specific, but rather to detect cadmium, lead, and/or mercury without differentiating between the three metals.

These studies demonstrate that biosensor technology is still relatively new for the detection heavy metal mixtures in water samples. As lead in drinking water remains a

major public health concern, biosensors specific for lead have been developed.

Previous studies provide limited background information about sampling parameters to consider in the design of future research due to the small numbers of samples, heavy metals in mixtures. The lack of testing in mixtures is a gap in our knowledge about the performance of biosensor technology.

The primary objective of this research is to evaluate the reliability and validity of a newly developed field-based biosensor methodology for the detection of heavy metal mixtures of cadmium and lead in drinking water through controlled laboratory testing and environmental sampling which will provide concentration values in real-time.

3. METHODS

3.1 **Background**

The scope for this work, *Analysis of Heavy Metal Exposures and Evaluation of Detection Methods for Heavy Metals in Drinking Water*, involves three research aims focused on exposure to heavy metals. Aim 1 focused on the analysis of hospital discharge data to evaluate heavy metal poisoning resulting in inpatient hospitalizations Illinois for years 2010-2013. Aims 2 and 3 concentrated on evaluating field-based methods of detection for specific heavy metals in drinking water. Aim 2 focused on testing the validity and reliability of a newly developed biosensor system methodology for the detection of cadmium and lead in drinking water. Aim 3 focused on testing the reliability of commercially available field kits for the detection of lead in drinking water.

3.2 **Methods for Hospitalizations from Heavy Metal Poisoning Cases in Illinois, 2010-2013**

3.2.1 **Data Sources**

The basis of the analysis was a billing database of outpatient and inpatient cases compiled and managed by the Illinois Hospital Association containing information about all patients treated in any Illinois hospital for any medical reason were analyzed. The outpatient database includes all patients treated in emergency rooms for less than 24 hours who were not admitted to the hospital in the years 2010-2013. The inpatient database included all the patients treated for 24 hours or more in any Illinois hospital for any medical reason for years 2010-2013. The variables included within the database fall into three main categories: patient demographics (age, sex, race/ethnicity),

exposure and health outcomes (diagnoses, hospital procedures, discharge status), and economics (hospital charges, payer source). Based on the annual state audit of hospitals, the hospitals included in the databases used for this analysis comprise 96.5% of all patient admissions statewide (Illinois Health Facilities and Services Review Board, 2011). Both the outpatient and inpatient cases were de-duplicated using date of birth, sex, race/ethnicity, patient zip, and hospital of treatment. The University of Illinois at Chicago (UIC) IRB has approved this work (#2012-0116).

3.2.2 Case Definition

A poisoning case was defined as a patient who had any diagnosis of heavy metal poisoning, which was determined by review of ICD-9-CM diagnosis codes, with up to 25 diagnosis codes for each patient, for reason for the visit (outpatient only) or the admission diagnosis (inpatient only), and cause of injury (all patients; ICD-9-CM external injury codes). All the fields were used to identify patients diagnosed with a heavy metal poisoning.

Patients were classified as being poisoned by any heavy metal, which included the following ICD-9-CM diagnosis codes (NCODES) and external injury codes (ECODES): 984.0 (toxic effect of lead and its compounds, including fumes: inorganic lead compounds), 984.1 (toxic effect of lead and its compounds, including fumes: organic lead compounds), 984.8 (toxic effect of lead and its compounds, including fumes: other lead compounds), 984.9 (toxic effect of lead and its compounds, including fumes: unspecified lead compounds), 985.0 (toxic effect of other metals: mercury and its compounds), 985.1 (toxic effect of other metals: arsenic and its compounds), 985.2

(toxic effect of other metals: manganese and its compounds), 985.3 (toxic effect of other metals: beryllium and its compounds), (toxic effect of other metals: mercury and its compounds), 985.4 (toxic effect of other metals: antimony and its compounds), 985.5 (toxic effect of other metals: cadmium and its compounds), 985.6 (toxic effect of other metals: chromium), 985.8 (other unspecified metal: brass fumes, copper salts, iron compounds, nickel compounds), and 985.9 (unspecified metal); E861.5 (accidental poisoning by lead paints), E866.0 (accidental poisoning by other and unspecified solid and liquid substances: lead and its compounds), E866.1 (accidental poisoning by other and unspecified solid and liquid substances: mercury and its compounds), E866.2 (accidental poisoning by other and unspecified solid and liquid substances: antimony and its compounds), E866.3 (accidental poisoning by other and unspecified solid and liquid substances: arsenic and its compounds), E866.4 (other metals and their compounds and fumes: beryllium (compounds, brass fumes, cadmium (compounds), copper salts, iron (compounds), manganese (compounds), nickel (compounds), and thallium (compounds)), E950.8 (suicide and self-inflicted poisoning by solid or liquid substances: arsenic and its compounds), and E980.8 (poisoning by solid or liquid substances, undetermined whether accidentally or purposely inflicted: arsenic and its compounds) (Table V). From this overall category, specific categories were created for each metal and associated compounds. The categories included the following ICD-9-CM codes: antimony (NCODE 985.4 and ECODE E866.2); arsenic (NCODE 985.1 and ECODES E866.3, E950.8, and E980.8); beryllium (NCODE 985.3); cadmium (NCODE 985.5); lead (NCODES 984.0-984.1, 984.8-984.9 and ECODE E866.0); mercury (NCODES 985.0 and ECODE E866.1); chromium (NCODE 985.6); manganese

(NCODE 985.2); and all other metals (NCODES 985.8-985.9, and ECODE E866.4). Additionally, a category specific to lead paint exposure was defined by the ECODE E861.5 (Table V).

A patient was considered to have a primary diagnosis of a poisoning when the primary diagnosis had a poisoning NCODE of N984.0-985.9 and considered to have a main diagnosis of a poisoning was when a patient had a primary diagnosis or first diagnosis of a poisoning or the reason for visit (outpatient) or reason for admission (inpatient) had a poisoning NCODE of N984.0-985.9. A routine discharge was defined as a patient who was discharged home or self-care and not discharged to another short-term hospital, skilled nursing facility, intermediate care facility, another type of institution, home health care service, or rehabilitation facility.

3.2.3 Statistical Analysis

To explore patterns among the poisoning cases, the following variables, in addition to the diagnosis codes, were considered: age, sex, race/ethnicity, length of stay, discharge status, geographic location (based on residential zip code), medical charges, and year. Total charges reflect all charges relating to the course of treatment, not just to the treatment of heavy metal poisoning(s). Chi-squared tests were calculated to determine if there were difference between outpatient and inpatient cases and age categories, sex, race/ethnicity, and discharge status. A t-test was conducted to determine if there were differences between total charges and patient type.

TABLE V
ICD-9-CM NCODES AND ECODES USED TO DETERMINE HEAVY METAL
POISONING CASES IN ILLINOIS

ICD-9-CM Code(s)	Description
984	Toxic effect of lead and its compounds (including fumes)
984.0	Inorganic lead compounds
984.1	Organic lead compounds
984.8	Other lead compounds
984.9	Unspecified lead compound
985	Toxic effect of other metals
985.0	Mercury and its compounds
985.1	Arsenic and its compounds
985.2	Manganese and its compounds
985.3	Beryllium and its compounds
985.4	Antimony and its compounds
985.5	Cadmium and its compounds
985.6	Chromium
985.8	Other specified metals: Brass fumes Copper salts Iron compounds Nickel compounds
985.9	Unspecified metal
E861.5	Accidental poisoning by lead paints
E866	Accidental poisoning by other and unspecified solid and liquid substances
E866.0	Lead and its compounds
E866.1	Mercury and its compounds
E866.2	Antimony and its compounds
E866.3	Arsenic and its compounds
E866.4	Other metals and their compounds and fumes: Beryllium (compounds) Brass fumes Cadmium (compounds) Copper salts Iron (compounds) Manganese (compounds) Nickel (compounds) Thallium (compounds)
E950	Suicide and self-inflicted poisoning by solid or liquid substances
E950.8	Arsenic and its compounds
E980	Poisoning by solid or liquid substances, undetermined whether accidentally or purposely inflicted
E980.8	Arsenic and its compounds

3.2.4 Hospitalization Incidence Rates

Incidence rates for the number of heavy metal poisoning cases resulting in hospitalizations were calculated. The crude incidence rate (IR) of the number of heavy metal poisoning cases was calculated as the number of cases per age group (age groups were in 5-year increments, based on the US Census age grouping), while the age-specific IRs were calculated as the number of heavy metal poisoning cases divided by the 2010 Illinois population, based on US Census data, for each age group, per 100,000 people (Equation 1).

$$IR = \left(\frac{\frac{\text{Number of Heavy Metal Poisonings per Age Group}}{2010 \text{ Illinois Census Population per Age Group}}}{100,000} \right) \quad (\text{Equation 1})$$

In order to calculate the 95% confidence interval (CI), the standard error, SE, was calculated first and was defined as the square root of the incidence rate, R, by one minus the incidence rate, R, divided by the total number of cases, N (Equation 2).

$$SE = \sqrt{\frac{R \times (1-R)}{N}} \quad (\text{Equation 2})$$

The 95% CI for the age-adjusted IR was calculated as the IR plus/minus the critical value for a 95% CI times by the standard error (Equation 3).

$$95\% \text{ CI: } IR \pm 1.96 \times SE \quad (\text{Equation 3})$$

A test for trend was conducted to determine if there was a trend in rates over the time period. SAS software (v 9.4, Cary, NC) was used for all statistical analyses.

3.2.5 National Survey Data

The number of heavy metal poisoning cases from this analysis was compared to the number of cases obtained from national health and surveillance data, specifically NHANES and AAPCC. NHANES data is publicly available data through CDC and the National Center for Health Statistics (NCHS). For the purposes of this project, the data from the CDC's *Fourth National Report on Human Exposure to Environmental Chemicals* was compared to the Illinois hospital data. This report is compilation of NHANES data from 2005-2006 through 2011-2012 survey years (Centers for Disease Control and Prevention, 2015a). The 2011-2012 survey year data was used as the comparison for this analysis. Biological measurement data was available for antimony, arsenic, beryllium, cadmium, lead, manganese, and mercury; however, biological measurements for chromium are not recorded by NHANES.

The AAPCC collaborates with the 55 poison centers in the US to track poisonings and their sources, including heavy metals (American Association of Poison Control Centers, 2015a). The National Poison Data System (NPDS) is the electronic health record collection system used by AAPCC member poison centers and collects data in real-time (American Association of Poison Control Centers, 2015b). The NPDS has national data as well as state-specific data. The number of cases for arsenic, cadmium, (trivalent) chromium, lead, manganese, mercury, elemental mercury, other metals and unknown metals were obtained.

3.2.6 Spatial Modeling

To determine whether there was spatial clustering among the heavy metal poisoning cases, geographic locations of the patients (zip code and county) and proximity to major highways, waterways, Superfund/National Priorities List(NPL) sites, Toxic Release Index (TRI) index and National Pollution Discharge Elimination System (NPDES) were considered. Superfund/NPL, TRI and NPDES data is publically available from US EPA. ArcGIS software (v 10.3, Redlands, CA) was used for all spatial analyses. To map potential spatial clusters, both Hot Spot Analysis (Getis-Ord Gi*) and Cluster and Outlier Analysis (Anselin Local Morans I) were used, with the results from the average nearest neighbor analysis used for the threshold distance.

3.3 Methods for Validity and Reliability of Biosensor Methodology for the Detection of Cadmium and Lead in Drinking Water

3.3.1 Biosensor System

The biosensor system evaluated in this research was manufactured by ANDalyze (Champaign, IL). The biosensor system consists of a handheld fluorimeter and a consumable (single-use) metal-specific sensor test kit, which are detailed below.

3.3.2 ANDalyze Sensors

The full details of the construction of the biosensors are described elsewhere (Lu, 2009). The following describes a brief overview of the biosensor are constructed. The metal-specific sensors are based on fluorescence and colorimetric sensor technology which uses in vitro selection, a combinatorial biology method, to obtain catalytic DNA with high specificity and selectivity for metal ions (Lu, 2009). The DNA is labeled with a

fluorophore/quencher pair and in the presence of metal ions, the catalytic DNA is cleaved, resulting in a dramatic increase of fluorescent signals (Lu, 2009). The metal-specific biosensors are highly selective and highly sensitive, with a limit of detection as low as 11 ppt (Lu, 2009). The cadmium sensor (model: AND016) has a detection range of 0.1 to 1.0 ppm (ANDalyze, 2013a). The lead sensor (model: AND010) has a detection range of 2 to 100 ppb (ANDalyze, 2013b). Each kit contains 25 single use metal-specific sensors (Figure 1) and cuvettes (Figure 2), 5 mL samples tubes with buffer, metal-specific standard solution, 1 mL syringes and 100 μ L fixed-volume pipette.



Figure 1: Metal-specific cadmium sensor.



Figure 2: Cadmium sensor with cuvette.

3.3.3 ANDalyze Fluorimeter

The handheld fluorimeter (Figure 3) utilized in this research was the AND1000 Fluorimeter, manufactured by ANDalyze (Champaign, IL). The fluorimeter allows for testing and data storage in a single, portable device (ANDalyze, 2013c). The fluorescence of the reaction is measured by the fluorimeter to determine the

concentration of the metal (ANDalyze, 2013c). The concentrations of the metals are reported in real-time, in less than two minutes (ANDalyze, 2013c). Step-by-step testing directions are provided in a detailed instruction manual and on the screen of the fluorimeter.



Figure 3: ANDalyze handheld fluorimeter (Model AND1000).

3.3.4 Standards

Stock solutions were purchased as a solution in 2% nitric acid (HNO_3) from SPEX CertiPrep, Custom Assurance Standard (Metuchen, NJ) and were serially diluted to the desired concentration. The cadmium standard was 11,240 ppm cadmium. The lead standard was 1000 ppm. Serial dilutions were prepared in deionized water and tap water for the four different known spiked concentrations of cadmium and four different known spiked concentrations of lead.

3.3.5 Water Samples and Sample Collection

Purified deionized (deionized) water was purchased from Thermo-Fisher Scientific (Hanover Park, IL), DNase- and Protease-free, sterile water. Deionized water was stored at room temperature, approximately 23.5°C (74.3°F). The pH of the deionized water was determined using the pH test strips provided by the manufacturer; pH was also validated with a pH meter. All samples were within the acceptable pH range of 5-8, as specified by the manufacturer.

Environmental drinking water samples (tap water) were obtained from a City of Chicago drinking water source. For tap water, water was flushed for ten minutes before collection and then stored at room temperature. Tap water samples were not analyzed until the water temperature was within the range of 20-25°C (68-77°F), specified as the most accurate and precise temperature for results by the manufacturer (ANDalyze, 2013b; ANDalyze, 2013c). All tap water samples were filtered with the ANDalyze filtration kit, according to the manufacturer's instructions.

3.3.6 Laboratory Analysis

3.3.6.1 Process for calibration

On-site calibration was required for each water sample. The manufacturer's instructions for on-site calibration were followed. Two separate samples, one spiked and one blank (not spiked), were required for the calibration. The provided sample tubes, with buffer, were filled to the 5 mL mark. In order to spike the sample for calibration, 100 µL of the provided metal standard solution from ANDalyze was pipetted into one of the samples, mixed with the samples, and allowed to stand for five minutes

before testing in the fluorimeter. Following the manufacturer's instructions: "Metal standard spiked for on-site calibration is required to incubate for all environmental water samples" (ANDalyze, 2013a; ANDalyze, 2013b). Once the sample had incubated, the fluorimeter was turned on and navigated to the "Site Calibration" program.

The cuvette was placed in the chamber of the fluorimeter with the sensor on top. With the provided syringe, 1 mL was drawn from the spiked sample and the syringe was attached to the sensor. The sample was ejected into the cuvette through the sensor, slowly over a three to five second time period. The sensor and syringe were removed from the top of cuvette, and the chamber door closed on the fluorimeter, with the directions of step 1 of 2 of the "Site Calibration" program followed as directed on the fluorimeter screen. Following the completion of step 1, the cuvette was removed from the fluorimeter chamber and a new cuvette and sensor were placed into the chamber. Using the same procedure for the spiked sample, 1 mL of the sample that was blank (not spiked) water was inserted into the cuvette. The resulting calibration factor was applied to the subsequent samples.

3.3.6.2 Analysis of Water Samples

The reliability and validity of the biosensor system was tested in a controlled laboratory setting. There were four different, known, spiked concentrations of cadmium and lead tested for both the deionized water and tap water, in addition to a blank (not spiked) water sample. Blank water samples of both deionized water and tap water were tested in order to establish baseline levels and determine the level of cadmium and lead in the sample.

Metal standards from SPEX CertiPrep were used to prepare the spiked samples and used throughout. The four cadmium standards were 0.05 ppm, 0.15 ppm, 0.25 ppm, and 0.50 ppm, based on the detection range of the sensor and the US EPA's MCL of 0.005 ppm (5 ppb) (United States Environmental Protection Agency, 2014b). For lead, the four standards were 5 ppb, 10 ppb, 15 ppb, and 25 ppb, which were based on the detection range for the sensor, the US EPA's TT AL and standard concentrations from a US EPA ETV report (United States Environmental Protection Agency, 2014d; Yates et al. 2013).

For both the deionized water and tap water, one sample of the cadmium standard and one sample of the lead standard were incubated at room temperature (23.5°C) and tested after three different time points (30 minutes, 60 minutes, and 120 minutes), to determine whether or not incubation time affects the concentrations detected by the biosensor system. Baghel et al. found that the greater the concentration, the more rapid the detection time for a rapid colorimetric method for the detection of arsenic (Baghel et al., 2007). Van Geen et al. found that when using the Hach EZ arsenic test kit, increasing the reaction time greatly increased the measurement accuracy (Van Geen et al., 2005).

The metal-specific analysis program was selected on the fluorimeter and the on-screen directions were followed. Calibration was completed for the blank (non-spiked) deionized and tap water samples and for each of the four cadmium standards and four lead standards. This process was described previously in section [3.3.6.1 Process for Calibration](#).

3.3.7 Quality Assurance and Quality Control

3.3.7.1 Water Sampling

Samples were quantified from triplicate samples, in sequence with positive and negative (blank) controls (Table VI and Table VII).

3.3.7.2 Sensors from Multiple Lots (Batches)

Samples were tested with sensors from three lots (batches). Each kit contains 25 sensors. One spiked concentration from both deionized water and tap water were reserved and tested with different lots to ensure reliability across the different sensor batches.

3.3.7.3 Water Quality Parameters

Turbidity, conductivity, and pH were tested in tap water samples, while these water quality samples can impact the accuracy of measured concentrations, only pH measurement is required prior to fluorimeter testing (Lu, 2009; Yates et al., 2013). Turbidity was tested using the Micro-TPI field Portable Turbidimeter (Infrared) for Turbidity Testing (HF Scientific, Fort Myers, FL) with all manufacturer's instructions followed for calibration and testing. Conductivity was measured with the Oakton CON 510 Benchtop Meter (Vernon Hills, IL) with all manufacturer's instructions followed for calibration and testing. For pH, the manufacturer provided pH strips were used.

TABLE VI
SUMMARY OF SAMPLING PARAMETERS FOR CADMIUM^a

Water Type^b	Cadmium Standard (ppm)	Water Temperature	Incubation Time
Deionized	0 (Negative Control)	Room	-
Deionized	0.05**	Room	60 minutes
Deionized	0.15	Room	60 minutes
Deionized	0.25	Room	60 minutes
Deionized	0.5	Room	30 minutes
Deionized	0.5	Room	60 minutes
Deionized	0.5	Room	120 minutes
Deionized	2 (Positive Control)	Room	60 minutes
Tap	0 (Negative Control)	Room	-
Tap	0.05**	Room	60 minutes
Tap	0.15	Room	60 minutes
Tap	0.25	Room	60 minutes
Tap	0.5	Room	30 minutes
Tap	0.5	Room	60 minutes
Tap	0.5	Room	120 minutes
Tap	2 (Positive Control)	Room	60 minutes

^a The lower limit of the detection limit for cadmium is 0.1 ppm. Since the MLC for cadmium (0.005 ppm) is well below the lower limit of detection, 0.05 ppm represents the MCL.

^b Each water sample as tested in triplicate with positive and negative controls.

TABLE VII
SUMMARY OF SAMPLING PARAMETERS FOR LEAD

Water Type^a	Lead Standard (ppb)	Water Temperature	Incubation Time
Deionized	0 (Negative Control)	Room	-
Deionized	5	Room	60 minutes
Deionized	10	Room	60 minutes
Deionized	15	Room	30 minutes
Deionized	15	Room	60 minutes
Deionized	15	Room	120 minutes
Deionized	25	Room	60 minutes
Deionized	100 (Positive Control)	Room	60 Minutes
Tap	0 (Negative Control)	Room	-
Tap	5	Room	60 minutes
Tap	10	Room	60 minutes
Tap	15	Room	60 minutes
Tap	15	Room	30 minutes
Tap	15	Room	60 minutes
Tap	15	Room	120 minutes
Tap	100 (Positive Control)	Room	60 Minutes

^a Each water sample was tested in triplicate with positive and negative controls.

3.3.7.4 ICP-MS Analysis, a Gold Standard Comparison

Samples were sent to the University of Chicago, Department of Chemistry, Mass Spectrometry Core Facility, an external laboratory, for ICP-MS analysis, which is still the gold standard method of detection for heavy metals in water. We followed the guideline provided by the external laboratory for the preparation of samples. Briefly, samples were prepared in 2% HNO₃ solution with 18 mega-Ohm (MΩ) doubly-deionized distilled water. Water samples were shipped on Thursday, March 10, 2016 to the University of Chicago within five hours of sample preparation, received on Friday, March 11, 2016, and analyzed on Monday, March 14, 2016. A 13-point calibration curve was used in an aqueous matrix of 2% HNO₃ (Poon, 2016). The counts per second were normalized against a tin (Sn) or bismuth (Bi) internal standard (Poon, 2016).

3.3.8 Statistical Analysis

3.3.8.1 Mean

As samples were tested in triplicate, mean measured concentrations by ANDalyze were calculated. The mean was calculated as the sum of three concentrations, denoted as C_1 , C_2 , and C_3 , from the triplicate samples divided by the sample size, N (Equation 4).

$$Mean = \frac{\sum C_1 + C_2 + C_3}{N} \quad (Equation\ 4)$$

For measured concentrations above or below the limit of detection (LOD), the mean concentration was calculated as the LOD divided by two (Equation 5).

$$Mean\ (above\ or\ below\ LOD) = \frac{LOD}{2} \quad (Equation\ 5)$$

3.3.8.2 Standard Deviation

The standard deviation (SD) quantifies the amount of variation within the data from the mean. The SD was calculated as the square root of the summation of the squared deviation of the values (measured concentrations) from the mean value divided by the sample size, N (Equation 6).

$$SD = \sqrt{\frac{\sum [(C_1 - Mean)^2 + (C_2 - Mean)^2 + (C_3 - Mean)^2]}{N}} \quad (Equation\ 6)$$

3.3.8.3 Coefficient of Variance

The coefficient of variance, CV, is a measure that describes the variability relative to the mean. The CV was calculated as the ratio of the SD to the mean (Equation 7).

$$CV = \frac{SD}{Mean} \quad (Equation\ 7)$$

3.3.8.4 Percent Error and Mean Percent Error

The percent error was calculated as the difference between the ANDalyze-measured and the expected concentration divided by the expected concentration and multiplied by 100% (Equation 8). Negative percent error indicates negative bias.

$$Percent\ Error = \frac{(Measured - Expected)}{(Expected)} \times 100\% \quad (Equation\ 8)$$

The mean percent error was calculated as the average percent error across the triplicate samples. Percent error and mean percent error were similarly calculated for the ICP-MS-measured concentrations relative to the expected concentration.

3.3.8.5 ANOVA Analysis

In order to determine whether there were differences among the expected cadmium and lead concentrations as compared to the measured concentrations and if there were differences among the expected and measured concentrations for cadmium and lead based on water type, two-way (factor) ANOVA with replication was conducted. The measured concentration was the dependent variable with the independent variables of expected concentration (degrees of freedom (DF) of five, the four standard concentrations, and the negative (blank) control and positive control), water type (DF of two) and the interaction of the expected concentration and water type (DF of five). SAS software (v 9.4, Cary, NC) was used for all statistical analyses.

3.4 Methods for Reliability of Commercially Available Field Kits for the Detection of Lead in Drinking Water with Lead and Lead-Mixtures

Three different commercially available lead detection field kits were purchased with the support of the University of Illinois at Chicago (UIC) Provost's Award. Kits were selected on usability (whether samples could be collected and/or tested by an individual in the general population), availability at home improvement stores and online retailers, endorsements by certified laboratories, state and/or local health departments, and news media outlets. In addition to the three commercially available field-based kits, a subset of samples was sent to an external lab for verification by ICP-MS analysis.

3.4.1 Background for First Alert Drinking Water Test Kit

The First Alert Drinking Water Test Kit is a commercially available, field-based (at-home) kit that tests for water quality based on US EPA standards (First Alert Trust, 2005). In addition to testing (dissolved) lead in drinking water, this kit also tests for bacteria, pesticides, nitrates, chlorine, and standard water quality parameters of pH and hardness (First Alert Trust, 2005). The First Alert Drinking Water Test Kit comes with the package insert with instructions for all the tests. The package insert states the following: "First Alert Drinking Water Test Kit is a screening test and can not be used to certify water as safe or unsafe for drinking. This test kit provides approximate results only when used in strict accordance with instructions. First Alert© expressly disclaims any liability resulting from the use of this product, failure to follow instructions, or reliance on test results" (First Alert Trust, 2005). However, the package insert also notes that if the water tests outside the US EPA MCL or guideline standards, contact the Safe Drinking Water Hotline, which is an information hotline contracted under the

US EPA (First Alert Trust, 2005; United States Environmental Protection Agency, 2016b). First Alert Drinking Water Test Kit is manufactured by First Alert Trust, BRK Brands, Inc. (Aurora, IL). First Alert reported an estimated annual sales of \$662 million, but specific sales data for the First Alert test kit was not available (Hoover's, Inc., 2016a).

3.4.2 Background for WaterSafe Water Test for Lead

The WaterSafe Water Test Kit for Lead is a commercially available field-based (at-home) test kit, largely sold at home improvement stores and online retailers. This test kit is specifically manufactured to test for lead. WaterSafe Water Test Kit for Lead is manufactured by DiscoverTesting.com, whose parent company is Silver Lake Research Corporation (Monrovia, CA) (DiscoveringTesting.com, 2015). According to the manufacturer's website, the WaterSafe Water Test Kit has been featured in several news media publications/outlets, including The Wall Street Journal, US News and World Report, People Magazine, Good Housekeeping Magazine, Women's Health, Popular Science, This Old House (TV show), and Alaska Airlines (DiscoverTesting.com, 2011a). Like the First Alert Drinking Water Test Kit, the WaterSafe test kit states that the kit is a screening tool and "cannot be used to certify water as safe or unsafe for drinking"; the test "provides approximate results ONLY when used in strict accordance with instructions" (DiscoverTesting.com, 2011b). Furthermore, the manufacturer, Silver Lake Research Corporation, "expressly disclaims any liability resulting from the use of this product, failure to follow the instructions, or reliance of test results"

(DiscoverTesting.com, 2011b). Silver Lake Research Corporation has an estimated annual sales of \$2.27 million (Hoover's Inc., 2016b).

3.4.3 Background for Pro-Lab Lead in Water Test Kit

The Pro-Lab Lead in Water Test Kit is a commercially available test kit that utilizes US EPA method 200.7 to measure lead in water, with a detection limit of 1 ppb (Pro-Lab, 2013). Unlike the First Alert Drinking Water Test Kit and the Watersafe Water Test Kit for Lead, this kit has the consumer collect the water sample and then mail it back to Pro-Lab analysis. The Pro-Lab Lead in Water Test Kit is manufactured by Professional Laboratories Inc. (Weston, FL), with reported annual sales of \$78 million (Hoover's Inc., 2016c).

3.4.4 Analysis of Samples

3.4.4.1 Water Samples

Purified deionized bottled water purchased from Thermo-Fisher Scientific (Hanover Park, IL), and stored at room temperature, approximately 23.5°C (74.3°F). Tap water samples were obtained from a City of Chicago drinking water source, in accordance with the manufacturer's instructions.

For the First Alert and WaterSafe kits, the cold tap was run for 15-20 seconds to flush standing water from pipes prior to testing (First Alert Trust, 2005; DiscoverTesting.com, 2011b). Water temperature was not specified by the manufacturer, so tap water was tested once the temperature was near room temperature, approximately 23.5°C (74.3°F).

For the Pro-Lab samples, the cold water tap was turned on to a slow trickle, with a plastic one-liter container placed underneath the faucet (Pro-Lab, 2013). Once the container was full, the water was stirred with a non-metal spoon (Pro-Lab, 2013). Water was then poured into the provided plastic test tube (Pro-Lab, 2013).

3.4.4.2 Standards

Standards used for spiking were purchased as stock solution in 2% HNO₃ from SPEX CertiPrep, Custom Assurance Standard (Metuchen, NJ) and serially diluted to the desired concentration. The lead standard was 1000 ppm, the copper standard was 1000 ppm, and the iron standard was 1000 ppm. Serial dilutions were prepared in both deionized and tap water.

3.4.4.3 Sampling Preparation

In order to establish background levels of lead in the water, blank samples of deionized water and tap water were tested. Deionized water was spiked with four different standards of lead, three different standards of lead-copper mixture, and three different standards of lead-iron mixture in triplicates. The four standards for lead tested in deionized water were 5 ppb, 10 ppb, 15 ppb, and 25 ppb. For tap water samples, seven standards for lead were tested: 5 ppb, 10 ppb, 13 ppb, 15 ppb, 17 ppb, 20 ppb, and 25 ppb. These standard concentrations were determined based on US EPA's TT AL for lead and the detection limit of the kit (United States Environmental Protection Agency, 2014a). For the lead-copper mixture, the standards tested were based on 15 ppb of lead (the US EPA TT AL) and three different concentrations of copper: 50 ppb,

approximate the average amount found in City of Chicago tap water (City of Chicago, 2015); 1300 ppb, the TT AL set by US EPA (United States Environmental Protection Agency, 2014a); and 2000 ppb. For the lead-iron mixture, the lead standard tested was 15 ppb of lead and three different concentrations of iron: 100 ppb, a trace amount; 300 ppb, the US EPA Secondary Drinking Water Standard and the concentration at which water can have visible discoloration (United States Environmental Protection Agency, 2016a); and 1000 ppb. Additionally, a positive control of a lead standard of 100 ppb was tested. The summary of sampling parameters for lead, lead-copper mixture, and lead-iron mixture for the First Alert and WaterSafe test kits are displayed below in Tables VIII through XI.

TABLE VIII
SUMMARY OF SAMPLING PARAMETERS FOR LEAD WITH FIRST ALERT
DRINKING WATER TEST KIT

Water Type^a	Lead Standard (ppb)
Deionized	0 (Negative Control)
Deionized	5
Deionized	10
Deionized	15
Deionized	25
Deionized	100 (Positive Control)
Tap	0 (Negative Control)
Tap	5
Tap	10
Tap	13
Tap	15
Tap	17
Tap	20
Tap	25
Tap	100 (Positive Control)

^a Each water sample was tested in triplicate with positive and negative controls.

TABLE IX
SUMMARY OF SAMPLING PARAMETERS FOR LEAD-COPPER MIXTURE WITH
FIRST ALERT DRINKING WATER TEST KIT

Water Type^a	Lead Standard (ppb)	Copper Standard (ppb)
Deionized	15	50
Deionized	15	1300
Deionized	15	2000
Tap	15	50
Tap	15	1300
Tap	15	2000

^a Each water sample was tested in triplicate with positive and negative controls.

TABLE X
SUMMARY OF SAMPLING PARAMETERS FOR LEAD-IRON MIXTURE WITH FIRST
ALERT DRINKING WATER TEST KIT

Water Type^a	Lead Standard (ppb)	Iron Standard (ppb)
Deionized	15	100
Deionized	15	300
Deionized	15	1000
Tap	15	100
Tap	15	300
Tap	15	1000

^a Each water sample was tested in triplicate with positive and negative controls.

TABLE XI
SUMMARY OF SAMPLING PARAMETERS FOR LEAD-COPPER MIXTURE WITH
WATERSAFE WATER TEST KIT FOR LEAD

Water Type^a	Lead Standard (ppb)	Copper Standard (ppb)	Iron Standard (ppb)
Tap	0 (Negative Control)	-	-
Tap	5	-	-
Tap	10	-	-
Tap	13	-	-
Tap	15	-	-
Tap	17	-	-
Tap	20	-	-
Tap	25	-	-
Tap	100 (Positive Control)	-	-
Tap	15	1300	-
Tap	15	-	300
Tap	15	1300	300

^a Each water sample was tested in sequence with positive and negative controls.

In total, 27 samples were collected and sent to Pro-Lab for lead analysis (samples were not tested in triplicated). One blank (non-spiked) deionized water sample and one blank (non-spiked) tap water sample were collected. For deionized water, four different standards of lead were submitted for testing: 5 ppb, 10 ppb, 15 ppb, and 25 ppb. For tap water samples, seven standards for lead were submitted for testing: 5 ppb, 10 ppb, 13 ppb, 15 ppb, 17 ppb, 20 ppb, and 25 ppb. For the lead-copper mixture, one sample of deionized water with 15 ppb of lead and 1300 ppb was sent for analysis. For the lead-iron mixture in deionized water, the mixture tested was 15 ppb of lead and 300 ppb of iron. A lead-copper-iron mixture of 15 ppb lead, 1300 ppb copper, and 300 ppb iron was sent for analysis. For the lead-copper mixture in tap water, all three mixtures were submitted for analysis: each contained 15 ppb lead and 50 ppb, 1300 ppb, and 2000 ppb copper. For the lead-iron mixture in tap water, all three mixtures were sent for analysis: each contained 15 ppb of lead, and 100 ppb, 300 ppb, and 1000 ppb iron. Lastly, three lead-copper-iron mixtures in tap water was sent for analysis, each with 15 ppb lead, 1300 ppb copper, and 100 ppb, 300 ppb, or 1000 ppb iron. Only single samples were sent to Pro-Lab for analysis. The summary of sampling parameters for lead, lead-copper mixture, and lead-iron mixture sent to Pro-Lab for analysis are displayed in Tables XII.

3.4.4.4 Procedure

Manufacturers' instructions were followed for each of the test kits. For the First Alert kits, using the provided dropper pipette and test vial, "two dropper-fuls of the water sample were placed into the test vial" (First Alert Trust, 2005). The contents of the vial

TABLE XII
SUMMARY OF SAMPLING PARAMETERS FOR LEAD, LEAD-COPPER MIXTURES,
AND LEAD-IRON MIXTURES WITH PRO-LAB LEAD IN WATER TEST KIT

Water Type ^a	Lead Standard (ppb)	Copper Standard (ppb)	Iron Standard (ppb)
Deionized	0 (Negative Control)	-	-
Deionized	5	-	-
Deionized	10	-	-
Deionized	15	-	-
Deionized	25	-	-
Deionized	100 (Positive Control)	-	-
Deionized	15	1300	-
Deionized	15	-	300
Deionized	15	1300	300
Tap	0 (Negative Control)	-	-
Tap	5	-	-
Tap	10	-	-
Tap	13	-	-
Tap	15	-	-
Tap	17	-	-
Tap	20	-	-
Tap	25	-	-
Tap	100 (Positive Control)	-	-
Tap	15	50	-
Tap	15	1300	-
Tap	15	2000	-
Tap	15	-	100
Tap	15	-	300
Tap	15	-	1000
Tap	15	1300	100
Tap	15	1300	300
Tap	15	1300	1000

^a Each water sample was tested in sequence with positive and negative controls.

were gently mixed (swirled) for several seconds and then placed onto a flat surface (First Alert Trust, 2005). The test strip for lead was then inserted into the test vial with the water sample (First Alert Trust, 2005). After ten minutes, the test strips were removed from the test vial and read (First Alert Trust, 2005). According to the manufacturer's instructions, a negative test is when the bottom line (next to the number 1) on the test strip is darker than on the top line (next to the number 2) and a positive test is when the top line of the test strip (next to the number 2) is darker than the bottom line (next to the number 1), or if there are equally dark lines (First Alert Trust, 2005). The manufacturer states that "if no lines appear, or both lines are very light, the test did not run properly and the result is not valid" (First Alert Trust, 2005).

For the WaterSafe kits, using the provided test vial and dropper pipette, one pipette-full of the sample was placed into the test vial (DiscoverTesting.com, 2011b). The test vial was swirled gently and then placed on a flat surface (DiscoverTesting.com, 2011b). The WaterSafe test strip was then inserted into the test vial, arrow pointed down, for ten minutes (DiscoverTesting.com, 2011b). After the ten minute test period, the test strips were removed from the test vial (DiscoverTesting.com, 2011b). The manufacturer indicates that the left line (next to the number 1 on the test strip) will be darker than the right line (next to the number 2 on the test strip) if the test is negative for lead (DiscoverTesting.com, 2011b). However, if the right line (next to the number 2 on the test strip) is darker than the left line (next to the number 1 on the test strip), then the test is positive (DiscoverTesting.com, 2011b). The WaterSafe kit states that "if no lines appear, or both lines are very light, the test did not run properly and the result is not valid" (DiscoverTesting.com, 2011b).

All the samples were sent to Pro-Lab within four hours of sample preparation and collection, within the 24 hours specified by the manufacturer. An email was received once the samples were received by Pro-Lab, with the results received within two weeks of the samples being received. According to Pro-Lab, the laboratory analysis used to analyze lead in water samples is inductively coupled plasma atomic emission spectrometry (ICP-AES), following EPA Method 200.7 (Pro-Lab, 2016).

3.4.4.5 Quality Assurance and Quality Control

To ensure quality assurance and quality control, all water samples (deionized and tap) were tested in triplicate, in sequence with positive and negative controls. Water samples were also sent to the University of Chicago, Department of Chemistry, Mass Spectrometry Core Facility, an external lab, for ICP-MS analysis, a gold standard method of detection for heavy metals in water. Preparation of samples was described previously in section [3.3.7.4 ICP-MS, a Gold Standard Comparison](#).

All First Alert and Pro-Lab kits were purchased at two separate times, February 19, 2016 and May 11, 2016, directly from the manufacturer. The first set of WaterSafe kits were purchased from a commercial online retailer on February 19, 2016 and a second set of kit was purchased on May 11, 2016, directly from the manufacturer. The expiration date for the First Alert kits was February 2017. The expiration date for the first set of WaterSafe kits was December 2016 and April 2018 for the second set of kits. The Pro-Lab Lead in Water Test Kits were purchased at two separate times (February 19, 2016 and May 11, 2016), directly from the manufacturer.

As mentioned previously, the First Alert Drinking Water Test Kits is designed to test for nitrates, bacteria, water quality parameters of pH, hardness and chlorine. The manufacturer's instructions were followed for each test parameter.

3.4.4.6 Statistical Analysis

3.4.4.6.1 True Positives, True Negatives, False Positives, and False Negatives

Since First Alert and WaterSafe yield binary response, we tabulated true and false positives and true and false negatives, and used these data to calculate sensitivity, specificity, false negative rate, false positive rate, precisions and accuracy, including negative (blanks) and positive controls. A true positive (TP) was when the test strip indicated positive result for a sample with a standard concentration that was at or above the manufacturer's detection limit of 15 ppb lead (the US EPA TT action level), whereas true negative (TN) was when the test strip indicated a negative result for a sample with a standard concentration that was below the detection limit of 15 ppb lead. A false positive (FP) was when the test strip indicated a positive results for a sample with a standard concentration that was below the manufacturer's detection limit of 15 ppb lead, and a false negative (FN) was when the test strip indicated a negative result for a sample with a standard concentration that was above the manufacturer's detection limit of 15 ppb lead. Test strips from the First Alert kits and the WaterSafe kits that resulted in an "error" reading were not included in the final performance measure calculations.

The FP rate was calculated as the number of FPs divided by the sum of the FPs and the TNs as shown in Equation 9.

$$\text{False Positive Rate} = \frac{FP}{FP + TN} \quad (\text{Equation 9})$$

The false negative rate was calculated as the number of FNs divided by the sum of the TPs and the FNs, as shown in Equation 10.

$$\text{False Negative Rate} = \frac{FN}{FN + TP} \quad (\text{Equation 10})$$

3.4.4.6.2 Sensitivity and Specificity

Sensitivity, or the true positive rate, is the proportion of positives that were correctly identified as positive. Sensitivity was calculated as the number of TPs divided by the sum of the number of TPs and FN (Equation 11).

$$\text{Sensitivity} = \frac{TP}{TP + FN} \quad (\text{Equation 11})$$

Specificity, or the true negative rate, is the proportion of negatives that were correctly identified as negative. Specificity was calculated as the number of TNs divided by the sum of the number of TNs and FPs (Equation 12).

$$\text{Specificity} = \frac{TN}{TN + FP} \quad (\text{Equation 12})$$

3.4.4.6.3 Accuracy and Precision

Accuracy is the proportion of the true results, both the TPs and TNs, among the total number of samples tested. Accuracy was calculated as the sum number of TPs and the number of TNs divided by the total number of TPs, FPs, TNs, FNs, or the sample size, N (Equation 13).

$$\text{Accuracy} = \frac{TP + TN}{TP + FP + TN + FN} \quad (\text{Equation 13})$$

Precision is a measure of how exact the results are; precision can be thought of as the repeatability, or the variation observed when the same person measures the same thing repeatedly with the same device. Precision was calculated as the number of TPs divided by the sum of the number of TPs and FPs (Equation 14).

$$Precision = \frac{TP}{TP + FP} \quad (Equation\ 14)$$

3.4.4.6.4 ANOVA Analysis

Since an actual concentration was provided from the Pro-Lab kit, TPs, TNs, FPs, and FNs were not calculated, rather two-way (factor) ANOVA analysis was conducted to determine if the measured concentration was different from the expected concentration, by water type, and the interaction of these two factors. The dependent variable was the measured concentration by Pro-Lab, with the independent variables the standard concentrations (DF of seven), water type (DF of one), and the interaction between the standard concentrations water type (DF of two). A coefficient of determination, R^2 , was also calculated to indicate the proportion of variance in the dependent variable (measured concentration by Pro-Lab) that is predicted from the independent variable (the standard concentration). SAS software (v 9.4, Cary, NC) was used for all statistical analyses.

3.4.4.6.5 Concordance Correlation Coefficient

A concordance correlation coefficient was calculated to evaluate inter-rater reliability for a subset of 14 samples of lead standards in tap water for both the First

Alert and WaterSafe test strips. Two scientists categorized the results of the test strips into three categories for each test strip – negative, positive, and error.

4. HOSPITALIZATIONS FROM HEAVY METAL POISONING CASES IN ILLINOIS, 2010-2013

4.1 Introduction

Exposure to heavy metals is a widespread public health issue today. Low levels of exposure to heavy metals are thought to be associated with a myriad of health effects, including adverse neurodevelopmental, dermatological, and cardiovascular effects and even cancer (Agency for Toxic Substances and Disease Registry, 2007b; Agency for Toxic Substances and Disease Registry, 2012). Though epidemiological studies indicate the occurrence of adverse health effects associated with heavy metals (Adams et al., 2012; Chung et al., 2014; Ferraro et al., 2010; Shankar et al., 2014).

It is unclear what the health and economic burdens of heavy metals exposures are, as existing surveillance systems are limited. Two key surveillance systems are the National Health and Nutrition Examination Survey (NHANES) and the National Poison Data System (NPDS). NHANES is a representative sample of the US population that includes biological measures of exposure to specific metals in the US population, but does not indicate health status associated with heavy metal exposures (Centers for Disease Control and Prevention, 2014). NPDS captures both acute and chronic poisonings from heavy metals reported by 55 poison control centers in the US, but does not indicate the nature of treatment received (Mowry et al., 2014).

There are no studies to date that analyze heavy metal exposures resulting in acute hospitalizations in the general populations. Because of the complexities involved with long-term biomonitoring, including biological measures of heavy metals and detailed information on potential sources of exposure, monitoring acute outcomes from poisonings due to heavy metal exposure offers an alternative method for surveillance of

exposure to heavy metals in the general population. In addition, hospital data captures cases that seek medical treatment for their exposures and provides clinical information regarding the acute treatment of these cases. In this analysis, all cases of heavy metal poisonings and cases associated with specific metals (antimony, arsenic, beryllium, cadmium, chromium, lead, lead paint, mercury, and other metals) are characterized using Illinois hospital discharge data from 2010-2013 to gain a comprehensive understanding of hospitalization relating to exposures to heavy metals resulting in poisonings. The main objective is to determine if hospital discharge data can be utilized as a surveillance tool to monitor heavy metal exposures in a general population. Secondary objectives were to assess economic burden of heavy metal poisonings and evaluate if there were spatial clustering among the cases.

4.2 Methods

4.2.1 Data Sources

The basis of the analysis was a billing database of outpatient and inpatient cases compiled and managed by the Illinois Hospital Association that contains information about all patients treated in any Illinois hospital for any medical reason. The outpatient database includes all patients treated in emergency rooms for less than 24 hours who were not admitted to the hospital in the years 2010-2013. The inpatient database included all the patients treated for 24 hours or more in any Illinois hospital for any medical reason in the years 2010-2013. The variables included within the database fall into three main categories: patient demographics (age, sex, race/ethnicity), exposure and health outcomes (diagnoses, hospital procedures, discharge status), and

economics (hospital charges, payer source). Based on the annual state audit of hospitals, the hospitals included in the databases used for this analysis comprise 96.5% of all patient admissions statewide (Illinois Health Facilities and Services Review Board, 2011). Both the outpatient and inpatient cases were de-duplicated using date of birth, sex, race/ethnicity, patient zip, and hospital of treatment. The University of Illinois at Chicago (UIC) IRB has approved this work (#2012-0116).

4.2.2 Case Definition

A poisoning case was defined as a patient who had any diagnosis of heavy metal poisoning, which was determined by review of ICD-9-CM diagnosis codes, with up to 25 diagnosis codes for each patient, for reason for the visit (outpatient only) or the admission diagnosis (inpatient only), and cause of injury (all patients; ICD-9-CM external injury codes). All the fields were used to identify patients diagnosed with a heavy metal poisoning.

Patients were classified as being poisoned by any heavy metal, which included the following ICD-9-CM diagnosis codes (NCODES) and external injury codes (ECODES): N984.0-984.1, N984.8-984.9, N985.0-985.6, and N985.8-985.9, E861.5, E866.0-E866.4, E950.8, and E980.8. Patients were also classified as being poisoned by a specific metal and/or its associated compounds. The metal-specific categories included the following ICD-9-CM codes: antimony (NCODE 985.4 and ECODE E866.2); arsenic (NCODE 985.1 and ECODES E866.3, E950.8, and E980.8); beryllium (NCODE 985.3); cadmium (NCODE 985.5); chromium (NCODE 985.6); lead (NCODES 984.0-984.1, 984.8-984.9 and ECODE E866.0); manganese (NCODE 985.2); mercury

(NCODES 985.0 and ECODE E866.1); and all other/unspecified metals (NCODES 985.8-985.9, and ECODE E866.4). Additionally, a category specific to lead paint exposure was defined as ECODE E861.5.

A patient was considered to have a primary diagnosis of a poisoning when the primary diagnosis had a poisoning NCODE of N984.0-985.9 and considered to have a main diagnosis of a poisoning was when a patient had a primary diagnosis or first diagnosis of a poisoning or the reason for visit (outpatient) or reason for admission (inpatient) had a poisoning NCODE of N984.0-985.9. A routine discharge was defined as a patient who was discharged home or self-care and not discharged to another short-term hospital, skilled nursing facility, intermediate care facility, another type of institution, home health care service, or rehabilitation facility.

4.2.3 Statistical Analysis

To explore patterns among the poisoning cases, the following variables, in addition to the diagnosis codes, were considered: age, sex, race/ethnicity, length of stay, discharge status, geographic location (based on residential zip code), medical charges, and year. Total charges reflect all charges relating to the course of treatment, not just to the treatment of heavy metal poisoning(s). Elixhauser comorbidity scale was used to calculate aggregated comorbidities (Elixhauser et al., 1998). Chi-squared tests were calculated to determine if there were difference between outpatient and inpatient cases and age categories, sex, race/ethnicity, and discharge status. A t-test was conducted to determine if there were differences between total charges and patient type. Age-specific and age-adjusted incidence rates with corresponding 95%

confidence intervals (CIs) were calculated for the number of heavy metal poisoning cases resulting in hospitalizations. A test for trend was conducted to determine if there was a trend in rates over the time period. SAS software (v 9.4, Cary, NC) was used for all statistical analyses.

4.2.4 Spatial Clustering

To determine whether there was spatial clustering among the heavy metal poisoning cases, geographic locations of the patients using residential zip codes and counties, along with proximity to major highways, waterways, and major industries were considered. ArcGIS software (v 10.2, Redlands, CA) was used for all spatial analyses. To map potential spatial clusters, both Hot Spot Analysis (Getis-Ord G_i^*) and Cluster and Outlier Analysis (Anselin Local Morans I) were used, with the results from the average nearest neighbor analysis used for the threshold distance.

4.3 Results

In total, there were 739 heavy metal poisoning cases treated in Illinois hospitals from 2010 through 2013, of which 437 (59%) were outpatient cases and 302 (41%) inpatient cases. Overall, 345 (47%) of all cases had a primary diagnosis of heavy metal poisoning, and 536 (72.5%) cases having a main diagnosis of heavy metal poisoning. Among inpatients cases, the majority (57.9%) had a length of stay of three or more days, with an average length of stay of 5.8 ± 14.5 days and a median length of stay of 3.0 (interquartile range: 2.0, 5.0). For inpatient heavy metal poisoning cases, only 140 (46%) had a routine discharge to their home.

There was no statistically significant spatial clustering (spatial autocorrelation $p>0.05$) of poisoning cases and rates of chromium, mercury, or overall based on geographic locations of the patients (zip code and county) and proximity to major highways, waterways, and major industries. There was statistically significant clustering among lead poisoning cases ($p<0.05$), but when standardizing for population (lead poisoning rate), the statistical significance of the spatial clustering decreased ($p>0.05$).

Demographic characteristics of poisoning cases for outpatient cases and inpatient cases are shown in Table XIII and Table XIV, respectively. The mean age of outpatient cases was slightly higher than for inpatient cases (37.4 ± 26.3 years and 34.0 ± 22.8 years, respectively). For both outpatient and inpatient cases, the largest proportion of cases were children under 18 years, comprising 32% of outpatients and 28% of inpatients, but nearly all of the children were treated for lead exposure only. When excluding lead exposure cases, the largest proportion of patients were over 65 years (14.5%). There was a statistically significant difference in age categories between outpatients and inpatients ($p<0.05$), but not by sex ($p=0.56$). A large proportion of the cases had exposure to multiple heavy metals (85%). The mean number metals to which patients were exposed was 2.4 ± 0.9 and 2.2 ± 0.7 for outpatients and inpatients, respectively. There a significant difference between outpatient and inpatient cases based on race, with higher number of White, non-Hispanic patients than Black/African American, Hispanic, Asian, and/or others ($p<0.05$). Additionally, there was a statistically significant difference between outpatients and inpatients and discharge status ($p<0.05$).

Overall, the cumulative hospital charges for outpatients was \$1,566,506 and \$10,620,573 for inpatients over the four-year period. The average total hospital charges among outpatients was \$3,585 USD (median of \$2,786.25) and \$35,284 USD among inpatients (median of \$18,146). Outpatients with arsenic poisonings had the highest median total charges of \$6,449, followed by cadmium (\$5,381) and chromium (\$4,708) (Table XIII). Inpatients with chromium poisonings had the highest median total charges of \$58,371, followed by arsenic (\$41,093), and mercury (\$21,734) (Table XIV). Outpatients and inpatients differed significantly for total hospital charges ($p < 0.05$).

Among all poisoning cases treated in Illinois hospitals between 2010 and 2013, the three most numerous heavy metal poisonings for patients 18 years or younger were lead (N=135), other/unspecified metals (N=62), and mercury (N=55). For patients between 18 to 44 years old, the three most numerous heavy metal poisonings were other/unspecified metals (N=132), mercury (N=89), and lead (N=23). The three most numerous heavy metal poisonings for patients between 45 and 64 years old were other/unspecified metals (N=152), mercury (N=81), and chromium (N=68). For patients 65 years and older, the three most numerous heavy metal poisoning cases were other/unspecified metals (N=101), chromium (N=59), and mercury (N=27).

For outpatients (Table XIII), the most common heavy metal poisonings were other/unspecified metals (N=276), mercury (N=167), chromium (N=115), and lead (N=88). For inpatients (Table XI), the most common heavy metal poisonings were other/unspecified metals (N=171), mercury (N=85), lead (N=83), lead paint (N=25), and chromium (N=18). With the exception of lead, all heavy metal poisoning categories had two or more exposures.

TABLE XIII
CHARACTERISTICS OF HEAVY METAL POISONING CASES TREATED IN ILLINOIS HOSPITALS AS OUTPATIENT CASES, 2010-2013

	Total Cases (N=437)	Arsenic (N=7)	Beryllium (N=5)	Cadmium (N=2)	Chromium (N=115)	Lead (N=88)	Lead Paint (N=3)	Manganese (N=2)	Mercury (N=167)	Other Metals (N=276)
Age in Years, Mean (SD)	37.4 (26.3)	53.4 (24.6)	30.4 (33.7)	77 (1.4)	62.7 (10.8)	9.4 (15.2)	1.3 (0.6)	34 (8.5)	38.3 (23.4)	47.2 (23.0)
Race/Ethnicity										
White, Non-Hispanic	304 (70%)	6 (86%)	2 (40%)	1 (50%)	106 (92%)	31 (35%)	1 (33%)	2 (100%)	127 (76%)	223 (81%)
Black/African American	64 (15%)	0 (0%)	1 (20%)	1 (50%)	5 (4%)	33 (8%)	1 (33%)	0 (0%)	14 (8%)	24 (9%)
Hispanic or Latino	35 (8%)	0 (0%)	2 (40%)	0 (0%)	1 (1%)	13 (15%)	1 (33%)	0 (0%)	13 (8%)	16 (6%)
Other	33 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	11 (13%)	0 (0%)	0 (0%)	13 (8%)	12 (4%)
Missing	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)
Sex										
Male	219 (50%)	5 (71%)	1 (20%)	2 (100%)	68 (59%)	47 (53%)	1 (33%)	1 (50%)	85 (51%)	131 (47%)
Female	217 (50%)	7 (29%)	4 (80%)	0 (0%)	47 (41%)	41 (47%)	2 (67%)	1 (50%)	82 (49%)	145 (53%)
Two of More Exposures	361 (83%)	7 (100%)	5 (100%)	2 (100%)	115 (100%)	12 (14%)	3 (100%)	2 (100%)	167 (100%)	276 (100%)
Primary Diagnosis	214 (49%)	2 (29%)	2 (40%)	0 (0%)	41 (36%)	56 (64%)	2 (67%)	0 (0%)	158 (95%)	100 (36%)
Main Diagnosis	339 (78%)	6 (86%)	5 (100%)	2 (100%)	84 (73%)	72 (82%)	2 (67%)	1 (50%)	164 (98%)	198 (72%)
Discharged Home	370 (85%)	6 (86%)	5 (100%)	2 (100%)	115 (100%)	82 (93%)	3 (100%)	2 (100%)	133 (80%)	229 (83%)
Median Total Hospital Charges (N, obs)	\$2,786 (437)	\$6,449 (7)	\$1,809 (5)	\$5,381 (2)	\$4,708 (115)	\$967 (88)	\$4,045 (3)	\$1,035 (2)	\$3,088 (167)	\$4,183 (276)

TABLE XIV
CHARACTERISTICS OF HEAVY METAL POISONING CASES TREATED IN ILLINOIS HOSPITALS AS INPATIENT CASES, 2010-2013

	Total Cases (N=302)	Arsenic (N=12)	Chromium (N=18)	Lead (N=83)	Lead Paint (N=25)	Manganese (N=1)	Mercury (N=85)	Other Metals (N=171)
Age in Years, Mean (SD)	34.0 (22.8)	35.9 (17.7)	62.2 (8.7)	12.8 (19.9)	2.0 (0.9)	46	38.2 (17.3)	43.7 (18.1)
Race/Ethnicity								
White, Non-Hispanic	178 (59%)	5 (42%)	18 (100%)	27 (33%)	8 (32%)	0 (0%)	52 (61%)	125 (73%)
Black or African American	66 (22%)	4 (33%)	0 (0%)	34 (41%)	10 (40%)	0 (0%)	15 (18%)	20 (12%)
Hispanic or Latino	32 (11%)	2 (17%)	0 (0%)	14 (17%)	4 (16%)	0 (0%)	11 (13%)	12 (7%)
Other	15 (5%)	1 (8%)	0 (0%)	5 (6%)	1 (4%)	1 (100%)	5 (6%)	8 (5%)
Missing	11 (4%)	0 (0%)	0 (0%)	3 (4%)	2 (8%)	0 (0%)	2 (2%)	6 (4%)
Sex								
Male	144 (48%)	8 (67%)	8 (44%)	48 (58%)	14 (56%)	1 (100%)	39 (46%)	73 (43%)
Female	158 (52%)	4 (33%)	10 (56%)	35 (42%)	11 (44%)	0 (0%)	46 (54%)	98 (57%)
Two or More Exposures	268 (89%)	12 (100%)	18 (100%)	49 (59%)	25 (100%)	1 (100%)	85 (100%)	171 (100%)
Primary Diagnosis	131 (43%)	5 (42%)	0 (0%)	55 (66%)	24 (96%)	0 (0%)	76 (89%)	42 (25%)
Main Diagnosis	197 (65%)	6 (50%)	11 (61%)	61 (73%)	24 (96%)	0 (0%)	78 (92%)	99 (58%)
Discharged Home	140 (46%)	6 (50%)	8 (44%)	61 (73%)	20 (80%)	1 (100%)	32 (28%)	53 (31%)
Median Total Hospital Charges (N, obs)	\$18,145 (301)	\$41,093 (12)	\$58,371 (18)	\$13,738 (83)	\$9,139 (25)	\$13,482 (1)	\$16,800 (84)	\$21,734 (171)

For both outpatients and inpatients, no poisoning cases required use of a ventilator or an operation. There were a total of 21 (4.8%) suicides among outpatients and 77 (25.5%) for inpatients. For outpatients, the top three payer types were Illinois Medicaid (26.8%), Medicare (25.6%), and other commercial insurance (19.5%). For inpatients, the top three payer types were Illinois Medicaid (29.8%), Medicare (23.8%), and other commercial insurance (18.9%). There was not a statistically significant difference between payor between outpatient and inpatient cases ($p>0.05$). There were six outpatient cases (1.4%) and two inpatient cases (1.0%) where the payor was workers' compensation.

An Elixhauser Comorbidity Index was used to assess comorbidities among poisoning cases. Overall, 48.8% of poisoning cases had at least one listed comorbidity, with uncomplicated hypertension (14.1%) as the most common comorbidity. For outpatients, 72.1% (N=315) poisoning cases had an Elixhauser score of zero compared to 20.9% (N=63) among inpatient cases. The mean Elixhauser score for outpatients was 0.45 ± 0.86 and 1.99 ± 1.76 for inpatients. The proportion of patients with neurological disorders was 3.2% (N=14) for outpatient cases and 9.6% (N=29) for inpatient cases. Approximately 3.0% (N=13) of outpatient cases and 8.6% (N=26) of inpatient cases had cardiac arrhythmias. Dialysis may potentially affect heavy metal exposures (Lee et al., 2016; Bailie et al., 2015; Hsu et al., 2015), but there were no cases that were receiving dialysis treatment and among outpatients and inpatients, renal failure was low (N=4, 0.9% and N=17, 5.6%, respectively). About 12.6% (N=38) of inpatient cases had deficiency anemia, but only 1.0% (N=4) of outpatient cases. Among outpatient and inpatient cases, 8.7% (N=38) and 23.5% (N=71) had depression,

respectively. The proportion of patients with liver disease was 1.0% (N=3) for outpatients, and 3.3% (N=10) for inpatients.

The annualized age-adjusted incidence rate among patients treated for heavy metal poisonings in Illinois hospitals for 2010, 2011, 2012 and 2013 were as follows: 0.7 cases per 100,000 (95% CI: 0.69, 0.70), 1.3 cases per 100,000 (95% CI: 1.29, 1.30), 1.8 cases per 100,000 (95% CI: 1.79, 1.80), and 2.0 cases per 100,000 (95% CI: 1.99, 2.00), respectively (Table XV). There was a significant increasing trend over time for the annualized age-adjusted incidence rates ($p < 0.05$).

4.4 Discussion

From 2010 through 2013, there was a total of 437 outpatient and 302 inpatient heavy metal poisoning cases treated in Illinois hospitals. The age-adjusted incidence rates for heavy metal poisonings showed a statistically significant increasing trend between 2010 and 2013 ($p < 0.05$), but because of the short-term period of follow-up it is unclear if this is capturing a long-term change resulting from increasing exposures or improved screening, or whether it is just a short-term aberration. Overall, there were no antimony cases and for inpatient cases, there were no cadmium inpatient poisoning cases. Children (18 years or younger) compromised 30.6% of cases, but they were predominately lead exposures. There were five cases of beryllium poisonings, which are expected to be exclusively occupational exposure cases, as beryllium is used in select industrial processes. Beryllium is a sensitizing agent, so occupational exposure limits are relatively low, and very low levels (0.03 ng/m³ in air and 0.002 ppb in water) are found in the environment (Agency for Toxic Substances and Disease Registry,

2002). Only two of the five cases, however, occurred in patients over the age of 18. It is unclear if the child exposure to beryllium resulted from carry home exposures by their parents who work with beryllium or were the result of environmental sources.

For both outpatients and inpatients, there were no reported poisoning cases for chromium in 2010 or 2011, but in 2012 and 2013 there was a sudden increase in the number of cases; for outpatients 50 cases in 2012 and 65 in 2013, and for inpatients, 10 cases in 2012 and 8 in 2013. The reason for this sudden increase is unknown. Some of the increase may be attributed to increased public awareness following news reports in August 2011 about elevated levels of hexavalent chromium in drinking water in Chicago, which may have translated to an increase in testing by medical professionals within hospitals (Hawthorne, 2011; Rodriguez, 2011). It should be noted that the highest proportion of chromium cases in 2012 and 2013 were in Peoria County (N=16, 12%), a county in central Illinois and relatively far from Chicago. In addition, the patient with chromium exposures were substantially older than the other heavy metal patients, average age over 60 years, and all cases of chromium exposures occurred concurrently with exposure to mercury or unspecified other heavy metals. This trend in increasing chromium poisoning cases in Illinois was not seen in the national AAPCC annual reports for 2010 through 2013 and this trend cannot be verified nationally, as NHANES does not test for chromium in their survey (Bronstein et al., 2010; Bronstein et al., 2011; Mowry et al., 2012; Mowry et al. 2013). Further investigation to identify the point source for these exposures is warranted based on their apparent specificity in time, space, and co-exposures among elderly patients.

TABLE XV
ANNUALIZED AGE-ADJUSTED INCIDENCE RATE PER 100,000 FOR ALL PATIENTS TREATED IN ILLINOIS
HOSPITALS FOR HEAVY METAL EXPOSURES IN BOTH OUTPATIENT AND INPATIENT SETTINGS, 2010-2013

Age Group	2010		2011		2012		2013		Total	
	Cases	Age-Specific Rate	Cases	Age-Specific Rate	Cases	Age-Specific Rate	Cases	Age-Specific Rate	Cases	Age-Specific Rate
00-04	11	1.3	43	5.1	37	4.4	37	4.4	128	15.3
05-09	7	0.8	11	1.3	10	1.2	12	1.4	40	4.7
10-14	3	0.3	2	0.2	1	0.1	7	0.8	13	1.5
15-19	9	1.0	18	2.0	16	1.7	16	1.7	59	6.4
20-24	6	0.7	5	0.6	13	1.5	8	0.9	32	3.6
25-29	8	0.9	9	1.0	13	1.4	11	1.2	41	4.5
30-34	6	0.7	15	1.7	6	0.7	10	1.2	37	4.3
35-39	5	0.6	4	0.5	13	1.5	12	1.4	34	4.0
40-44	6	0.7	15	1.7	16	1.8	10	1.1	47	5.4
45-49	7	0.7	9	1.0	14	1.5	18	1.9	48	5.1
50-54	8	0.9	9	1.0	28	3.0	12	1.3	57	6.1
55-59	2	0.2	9	1.1	11	1.4	27	3.3	49	6.1
60-64	2	0.3	5	0.8	16	2.4	16	2.4	39	5.9
65-69	3	0.6	3	0.6	12	2.5	23	4.7	41	8.5
70-74	0	0.0	6	1.6	9	2.5	19	5.2	34	9.3
75-79	1	0.3	1	0.3	10	3.5	9	3.1	21	7.3
80-84	1	0.4	3	1.3	0	0.0	10	4.2	14	5.9
85+	1	0.4	0	0.0	3	1.3	1	0.4	5	2.1
Total	86	0.7	167	1.3	228	1.8	258	2.0	739	5.7

Although there was a statistically significant difference between outpatient and inpatient cases based on race/ethnicity ($p < 0.05$) and discharge status ($p < 0.05$), 25% of the race/ethnicity categories and 69% of the categories for discharge status had a small sample size ($N < 5$), so the Chi-squared test may not be valid. There was not a statistically significant difference between outpatient and inpatient cases based on payor type ($p > 0.05$), but 54% of payor categories had small sample size ($N < 5$), so the Chi-squared test may not be valid.

There were more cases identified as general lead poisoning cases rather than being identified as having exposures from paint contaminated with lead, which has a different ICD-9-CM code. All lead paint poisonings occurred in children under 18 years of age, and nearly all the lead poisonings that did not identify paint as the main source of exposure occurred in children as well (79.0%; 135 of 171 cases). Additionally, for both outpatients and inpatients, all heavy metal categories had two or more exposures, with the exception of lead. In Illinois, of the children that were tested, there was a total of 1,701 (1.1%) confirmed cases of children who had blood lead levels greater than 10 $\mu\text{g/dL}$ in 2013 (Centers for Disease Control and Prevention, 2016a).

4.4.1 Comparison to National Survey Data

There are currently two primary surveillance tools that can be used to examine exposures to heavy metals in the general population – NPDS and NHANES. Between 2010 and 2013, the AAPCC recorded the following number of single exposures to heavy metals in Illinois: 63 arsenic, 9 cadmium, 330 lead, 3 manganese, 10 mercury,

125 elemental mercury, 275 other, and 3 unknown (National Poison Data System, 2016).

Because nearly all the lead exposure cases occurred in children, it is unlikely to be of an occupational source. With the recurring issues of environmental contamination and the susceptibility of children to lead's adverse health effects it is important to invest in more robust surveillance of lead poisoning in children. Many states only require a single lead test for children before entering kindergarten, at which point it may already be too late to prevent adverse developmental effects. The robust literature demonstrates the impact adverse cognitive and behavioral effects at low doses at earlier ages, with lead having a slow clearance from the body, with half-life of 30 days in blood, but 27 years in bone (Bellinger et al., 1991; Canfield et al., 2003; Evens et al., 2015; Agency for Toxic Substances and Disease Registry, 2007b). However, despite these adverse effects, there are currently only 29 funded states for child lead surveillance programs and limited funding for Adult Blood Lead Epidemiology and Surveillance (ABLES) (Centers for Disease Control and Prevention, 2016b; Centers for Disease Control and Prevention, 2015b).

Poison center data provides comprehensive information of both aggregate and substance specific exposures, in addition to dose, severity, and outcomes; however, poison center data comprises primarily of minor exposures not involving adverse health effects that require treatment in health care facilities. In contrast, national health surveys capture patients treated in outpatient and inpatient settings (Web-based Injury Statistics and Query and Reporting System, 2012; National Health Interview Survey, 2012; National Hospital Discharge Survey, 2011; Ambulatory Health Care Data, 2009).

Without having the biological measure from the hospital discharge data, the comparisons to NHANES data is more complex, as making determination of what is considered a poisoning directly from the NHANES data may create misclassification of poisoning cases, since NHANES only reports the measure level for heavy metals in the body, not if the person suffered adverse health effects from these exposures. Thus, utilizing hospital discharge data could help to fill the gap of national surveillance data.

4.4.2 Limitations

The determination of a heavy metal poisoning was based on ICD-9-CM codes. Since ICD-9-CM codes are for the purposes of coding/billing, there is no information provided on dose; thus, quantity of the heavy metal, laboratory confirmation of the presence of heavy metal(s), timing of the initial exposure, the frequency of exposure, and timing of laboratory findings is not available. Additionally, the use of ICD-9-CM codes may be missing heavy metal poisoning cases because the codes may reflect the clinical presentation and outcome, rather than the underlying causative agent. Because of this, it is difficult to determine how exposure to heavy metal(s) played a role in the diagnosis, treatment, and/or prognosis of a patient. Furthermore, because the fundamental goal of a billing dataset is to acquire the highest rate of reimbursement for the health care facility, the diagnoses listed for a patient may not accurately reflect the treatment or immediate threat to life. The combination of these factors relating to the use of billing datasets may result in misclassification and/or omission of heavy metal poisoning cases (Friedman et al., 2014; Krajewski and Friedman, 2015).

Additionally, the hospital discharge data does not capture data on the source of exposure or biological measurements in the body for heavy metals. This makes it difficult to discern patients being screened for heavy metal exposures from those being treated for adverse effects from these exposures. However, based on our data the majority had a primary diagnosis or reason for a visit related to heavy metal exposures. This indicates, that at least among the inpatient cases, these are likely cases being treated for adverse health effects related to heavy metal exposure. But the source is unknown. The exposure to heavy metals could be from occupational sources which seems most likely for adults, but because the zip code information is based on the patient's residence, it is hard to characterize spatial clustering in relation to known worksites or distinguish between combined environmental and occupational sources (Agency for Toxic Substances and Disease Registry, 2007b).

4.5 Conclusion

Hospital discharge data has not previously been utilized for surveillance of heavy metals exposures resulting in hospitalizations in the general population. However, there are some limitations with hospital discharge data as a surveillance tool. Although the hospital data captures exposure, health outcomes (diagnoses, hospital procedures, length of stay, discharge status), and economics (hospital charges, payer source), which are not recorded in national health survey data, it does not provide biological measurements nor the source of exposure for the heavy metals. Hospital discharge data likely captures predominately the most severe or acute cases of exposure to heavy metals rather than chronic or low-dose exposures that do not result in signs or

symptoms,, possibly from environmental sources. However, using billing data in conjunction with other surveillance tools will help build a broader picture of potential heavy metal exposures in a population and possibly identify “outbreaks” within the population. The economic burden of heavy metal poisonings resulting in treatment at a hospital is substantial in Illinois alone and having data sources of exposures available would help in identifying geographical areas or occupational settings where exposures should be mitigated. This information could in turn help environmental public health officials and industries to monitor and ensure compliance to reduce the burden of heavy metal poisonings resulting in hospitalizations.

5. VALIDITY AND RELIABILITY OF FIELD-BASED BIOSENSOR METHODOLOGY FOR THE DETECTION OF CADMIUM AND LEAD IN DRINKING WATER

5.1 Introduction

Exposure to heavy metals remains a leading public health concern due to the ubiquity and accumulation of metals in the environment. Even at low concentrations, heavy metals have been known to cause adverse effects including neurodevelopmental effect, renal effects and even cancer (Agency for Toxic Substances and Disease Registry, 2007b; Agency for Toxic Substances and Disease Registry, 2012). Contaminated drinking water is a common source of exposure to low levels of heavy metals, especially in the United States (US). However, challenges remain in identifying and measuring heavy metals in drinking water supplies at extremely low concentrations; while drinking water treatment plants may have access to sophisticated analytical methods, there is a need for field-based analytical methods that provide information at low-cost in real-time (Villanueva et al., 2014).

Field-based methods for the detection of heavy metals in drinking water have become more readily available in recent years. While some field-based methods have been found to be reliable, many have been tested for the performance against a single heavy metal, most commonly arsenic, and in areas where concentration in drinking water are known to be elevated (Rahman et al., 2002; Van Geen et al., 2005; Jakariya et al., 2007; Baghel et al., 2007). Previous studies field-based methods are not sensitive enough to measure concentrations at/near the US Environmental Protection Agency's (US EPA) maximum contaminant levels for arsenic (Rahman et al., 2002; Van Geen et al., 2005; Jakariya et al., 2007; Baghel et al., 2007; Sankararamakrishnan et al., 2008). Additionally, the majority of field tests are based on color chart matching

(colorimetric methods), which relies on subjective color identification to provide an estimated concentration or concentration range. The subjectivity of the results and interpretation can be problematic for the user in determining what concentration range the metal falls into or whether or not the metal is present or absent.

Biosensors are one analytical method with the potential to quantify one or more heavy metals in real-time at concentrations near MCLs or TT action levels, and provide actual concentration readings rather than indicate the presence of a heavy metal above a threshold level. A biosensor consists of immobilized biological material in intimate contact with a compatible transducer, which will convert the biochemical signal into a quantifiable electrical signal (Rategarpanah et al., 2013). The construction of biosensors may vary depending on the proposed contaminant of detection, but commonly rely on analysis of gene expression, utilizing a promoter of interest and a reporter gene (Raja and Selvam, 2011; Rategarpanah et al., 2013). The quantity of gene expression is a measure of the availability of a specific pollutant in the environment (Raja and Selvam, 2011; Rategarpanah et al., 2013). Although biosensors for heavy metals have been developed and successfully tested in controlled laboratory settings (Raja and Selvam, 2011; Rategarpanah et al., 2013; Cerminati et al., 2015; Chu et al., 2015; Li et al., 2015), they have not been widely tested in field conditions or widely mass marketed.

The primary goal of this research was to evaluate the validity and reliability of a commercially-available field-based biosensor methodology, ANDalyze, for the detection of the heavy metals cadmium and lead in drinking water. ANDalyze was selected for testing because of its capabilities to measure multiple metal-specific analytes

quantitatively in real-time, and because it is one of the only mass marketed systems that can detect both cadmium and lead. Cadmium was selected because it is a non-essential metal that is toxic to humans. Lead was selected because it is ubiquitous in drinking water and causes well-established adverse health outcomes, especially in children. Furthermore, following the issues in Flint, MI, there is growing awareness and concern over the presence of lead in drinking water. A laboratory-based approach was used, in which purified deionized bottled water and drinking water from a tap were spiked with known concentrations of cadmium or lead and measured using ANDalyze. A conventional laboratory-based detection method, inductively coupled plasma mass spectrometry (ICP-MS) was used to verify the spiked concentrations.

5.2 Methods

5.2.1 Field-Based Biosensor System

The ANDalyze (Champaign, IL) biosensor system is described in detail elsewhere (Lu, 2009). Briefly, it consists of a handheld fluorimeter and a consumable (single-use) metal-specific sensor test kit that contains 25 single use metal-specific sensors and cuvettes, 5 mL sample tubes with buffer, metal-specific standard solution, 1 mL syringes, and 100 μ L fixed-volume pipette. The handheld fluorimeter (model: AND1000) allows for testing and data storage in a single, portable device. The fluorescence of the reaction is measured by the fluorimeter to determine the concentration of the metal (ANDalyze, 2013a).

The metal-specific sensors are based on fluorescent and colorimetric sensor technology uses in vitro selection, a combinatorial biology method, to obtain catalytic

DNA with reportedly high specificity and selectivity for the target metal ion (Lu, 2009). The DNA is labeled with a fluorophore/quencher pair and in the presence of the target metal ion, the catalytic DNA is cleaved, resulting in a dramatic increase of fluorescent signals (Lu, 2009).

Metal-specific sensors for cadmium and lead were tested in this analysis. Based on the manufacturer's documentation, the cadmium sensor (model: AND016) has a detection range of 0.1 to 1.0 ppm (ANDalyze, 2013b) and the lead sensor (model: AND010) has a detection range of 2 to 100 ppb (ANDalyze, 2013c).

5.2.2 Experimental Design and Analysis

5.2.2.1 Calibration

Following the manufacturer's instructions for on-site calibration, calibration was performed prior to analysis of every sample using a standard (spiked sample) and a blank. The metal-specific standard solution provided in the kit was used. Once the sample had incubated for the specified five minute period, the fluorimeter was turned on and navigated to the "Site Calibration" program. The cuvette was placed in the chamber of the fluorimeter with the metal-specific sensor on top. With the provided syringe, 1 mL was drawn from the spiked sample and the syringe was attached to the sensor. The sample was injected into the cuvette through the sensor over a three to five second time interval. The sensor and syringe were removed from the top of the cuvette, and the chamber door closed on the fluorimeter. Once the fluorimeter analyzed the sample, the second sample, the blank water sample was analyzed, using the same

procedure. From the calibration, the resulting calibration factor is applied to the subsequent samples.

5.2.2.2 Preparation of Samples

Two different water sources were used: purified, deionized bottled water (deionized water) and environmental drinking water (tap water) from the City of Chicago. The deionized water was stored at room temperature, at approximately 23.5°C (74.3°F). Tap water was collected after ten minutes of flushing and was stored at room temperature; all tap water used in this study was collected at two different time periods, August and October 2015, from the same location. All samples were 20-25°C (68-77°F) at the time of analysis, as specified by the manufacturer for optimal results (ANDalyze, 2013a; ANDalyze, 2013b). All tap water samples were filtered using the ANDalyze filtration kit, following the manufacturer's instructions.

Blank water samples of both deionized water and tap water were tested in order to establish background levels, and to serve as a negative control. Four standards of cadmium (0.05 ppm, 0.15 ppm, 0.25 ppm, and 0.50 ppm) and lead (5 ppb, 10 ppb, 15 ppb, and 25 ppb) were made in both deionized water and tap water using stock solutions in 2% nitric acid (HNO₃) from SPEX CertiPrep, Custom Assurance Standard (Metuchen, NJ). The four cadmium standards were based on the detection range of the sensor and trace levels found in the environment (Agency for Toxic Substances and Disease Registry, 2012c). The lower limit of detection for the cadmium sensor is 0.1 ppm, which is 20 times the MCL of 0.005 ppm for cadmium in drinking water; for this reason, we were not able to test concentrations at the MCL. For lead, the four

standards were based on the detection range for the sensor, US EPA's treatment technique action level for lead (United States Environmental Protection Agency, 2014b). The cadmium stock solution was 10^{-1} M (11,240 ppm) cadmium and the lead stock solution was 1,000 ppm. In addition, positive controls were made in both deionized water and tap water, spiked to 2.0 ppm cadmium or 100 ppb lead and analyzed with the metal standards for cadmium and lead.

5.2.2.3 Experimental Design

Each of four known concentrations for cadmium and the four known concentrations for lead were analyzed in triplicates, with a negative control and a positive control. Turbidity, conductivity, and pH of deionized water and tap water were measured, as these factors can impact the accuracy and precision of measured concentration (Lu, 2009). Turbidity was tested using the Micro-TPI field Portable Turbidimeter (Infrared) for Turbidity Testing (HF Scientific, Fort Myers, FL) with all manufacturer's instructions followed for calibration and testing. Conductivity was measured with the Oakton CON 510 Benchtop Meter (Vernon Hills, IL) with all manufacturer's instructions followed for calibration and testing. For pH, the manufacturer provided pH strips were used. These water quality parameters were obtained prior to spiking.

For both the deionized water and tap water, prior to adding the sample in the provided 5 mL buffer tubes, one sample of a known concentration of 0.5 ppm cadmium and one sample of a known concentration of 15 ppb lead were incubated at room

temperature (23.5°C) for three different times (30 minutes, 60 minutes, and 120 minutes) to see if time affects the concentrations detected by the biosensor system.

5.2.2.4 ICP-MS Analysis

Samples of the four standards of cadmium and lead and blanks of deionized water and tap water were sent to an external lab (University of Chicago, Department of Chemistry, Mass Spectrometry Core Facility) for ICP-MS analysis. The lower limit of detection for cadmium and lead was 0.1 ppb. Samples were prepared in 2% HNO₃ solution with 18 mega-Ohm (MΩ) doubly-deionized water. Samples were shipped within five hours of sample preparation, received the next day and were analyzed the following business day. We followed the guidelines provided by the external laboratory for the preparation of samples.

5.2.3 Statistical Analysis

As samples were tested in triplicate, the mean measured concentrations, standard deviations, and coefficient of variance (CV) were calculated from ANDalyze results. For measured concentrations from ANDalyze that were below the lower limit of detection (0.1 ppm for cadmium and 2 ppb for lead), the result was replaced by half the limit of detection. For measured cadmium concentrations from ANDalyze that were above the upper limit of quantification (1.0 ppm for cadmium and 100 ppb for lead), the result was replaced by the upper limit of quantification. This occurred 23 times for the cadmium samples, and six times for the lead samples.

Percent error was calculated as the difference between the ANDalyze-measured and the expected concentration divided by the expected concentration and multiplied by 100%. Negative percent error indicates negative bias. The mean percent error was calculated as the average percent error across the triplicate samples. Percent error and mean percent error were similarly calculated for the ICP-MS-measured concentrations relative to the expected concentration. In order to determine whether there were systematic differences in ANDalyze quantification of cadmium and lead concentrations relative to standard concentration based on water type, two-way (factor) ANOVA with replication was conducted. The measured concentrations was the dependent variable with the independent variables of standard concentration (degrees of freedom (DF) of five, the four standard concentrations, and the negative (blank) control and positive control), water type (DF of two) and the interaction of the standard concentration and water type (DF of five). Lastly, a coefficient of determination, R^2 , was calculated. SAS software (v 9.4, Cary, NC) was used for all statistical analyses.

5.3 Results

All water samples had a temperature of approximately 20.8°C, with a pH of 6 for the deionized water and a pH of 7 for tap water prior to spiking, which is within the specified range of the manufacturer and is the only water parameter that ANDalyze has a specified range for testing. For tap water samples, the conductivity was 314 micro-Siemens (μS) and the turbidity was 0.3 Nephelometric Turbidity Units (NTU). The ICP-MS results confirmed that the standards were made as expected (Table XVI). ANDalyze tested and reported the levels for 15 chemicals that resulted in a $\pm 10\%$

change in the measured lead concentration: our ICP-MS analysis quantified six of these metals (cadmium, cooper, iron, lead, mercury, and zinc) and found that all were present below the interference level.

TABLE XVI
ICP-MS ANALYSIS RESULTS FOR CADMIUM AND LEAD

Cadmium Standard (ppm)	ICP-MS Analysis Result for Cadmium(ppm)^a	Percent Error
0.00 (Blank)	<0.1	-
0.05	<0.1	-
0.15	0.02	-87%
0.25	0.23	-8%
0.50	0.46	-8%
Lead Standard (ppb)	ICP-MS Analysis Result for Lead (ppb)^a	Percent Error
0 (Blank)	<0.1	-
5	4.0	-20%
10	10.3	3%
15	16.7	11%
25	28.2	13%

^a The lower limit of detection for ICP-MS was 0.1 ppb

5.3.1 Cadmium Samples

ANDalyze results for cadmium concentrations in deionized water show the method is consistently positively biased with mean percent errors between 33% and 120% (Table XVII). The method correctly identified five of six samples (83%) to be below the method of detection, 0.1 ppm, and correctly identified the 2 ppm standard samples to be in excess of the upper quantification limit, 1 ppm. Increasing incubation time for the 0.05 ppm standard increased the likelihood the method was positively biased: after 30 minute incubation period, the method had mean percent error of 43%,

but for the 60 minute and 120 minute incubation periods, the method indicated all samples were above the upper quantification limit.

In tap water, similar to in deionized water, ANDalyze correctly identified six samples below the method detection limit, 0.1 ppm, and correctly identified the 2 ppm standard samples to be in excess of the upper quantification limit, 1 ppm (Table XVIII). Results, however, were inconsistent for the 0.15 ppm and 0.25 ppm standards in tap water, sometimes showing positive bias and other times showing negative bias (percent error -100% to 567%), but mean percent errors were positive. All of the results for the 0.5 ppm standard incorrectly identified the samples as being in excess of the upper limit of quantification, so it is not possible to evaluate the impact of incubation time on method performance in tap water (Table XVIII).

5.3.2 Lead Samples

ANDalyze correctly identified the blank samples of deionized water as containing lead concentration below the method limit of detection, 2 ppb (Table XIX), and correctly identified standards of 100 ppb as being at or above the method upper limit of quantification, 100 ppb. For sample in the range of 5-25 ppb lead in deionized water, the ANDalyze method was consistently negatively biased, with mean percent errors ranging from -33% to -67% (Table XIX). Increasing the incubation time of the 15 ppb standard in deionized water, deionized the ANDalyze performance, with mean percent error moving from -51% to -67%.

TABLE XVII
SUMMARY OF RESULTS FOR MEASURED CADMIUM FROM ANDALYZE FOR
DEIONIZED WATER

Cadmium Standard (ppm)	Result from ANDalyze (ppm)	Mean±SD ^a (ppm)	CV (%)	Percent Error	Mean Percent Error	Incubation Time (minutes)
0.0 (Blank)	<0.1 <0.1 <0.1	0.05±0	-	-	-	-
0.05	0.1 <0.1 <0.1	0.07±0.03	43%	100% 0% 0%	33%	60
0.15	0.21 0.18 0.24	0.21±0.03	14%	40% 20% 60%	40%	60
0.25	0.60 0.57 0.48	0.55±0.06	11%	140% 128% 92%	120%	60
0.50	0.75 0.69 0.71	0.72±0.03	4%	50% 38% 42%	43%	30
0.50	>1.0 >1.0 >1.0	1.0±0	-	100% 100% 100%	100%	60
0.50	>1.0 >1.0 >1.0	1.0±0	-	100% 100% 100%	100%	120
2.00	>1.0 >1.0 >1.0	1.0±0	-	100% 100% 100%	100%	60

^a For measured concentrations below the limit of detection, a mean value was calculated as half the limit of detection. For measured concentrations above the limit of detection, the upper limit of detection was used for to calculate the mean value.

TABLE XVIII
SUMMARY OF RESULTS FOR MEASURED CADMIUM FROM ANDALYZE FOR TAP WATER

Cadmium Standard (ppm)	Result from ANDalyze (ppm)	Mean±SD^a (ppm)	CV (%)	Percent Error	Mean Percent Error	Incubation Time (minutes)
0.0 (Blank)	<0.1 <0.1 <0.1	0.05±0	-	-	-	-
0.05	<0.1 <0.1 <0.1	0.05±0	-	0% 0% 0%	0%	60
0.15	>1.0 0.36 <0.1	0.47±0.48	102%	567% 140% -100%	202%	60
0.25	>1.0 <0.1 <0.1	0.37±0.55	1%	300% -80% -80%	47%	60
0.5	>1.0 >1.0 >1.0	1.0±0	-	100% 100% 100%	100%	30
0.5	>1.0 >1.0 >1.0	1.0±0	-	100% 100% 100%	100%	60
0.5	>1.0 >1.0 >1.0	1.0±0	-	100% 100% 100%	100%	120
2.00	>1.0 >1.0 >1.0	1.0±0	-	100% 100% 100%	100%	60

^a For measured concentrations below the limit of detection, a mean value was calculated as half the limit of detection. For measured concentrations above the limit of detection, the upper limit of detection was used for to calculate the mean value.

TABLE XIX
SUMMARY OF RESULTS FOR MEASURED LEAD FROM ANDALYZE BY
DEIONIZED WATER

Lead Standard (ppb)	Result from ANDalyze (ppb)	Mean±SD ^a (ppb)	CV (%)	Percent Error	Mean Percent Error	Incubation Time (minutes)
0 (Blank)	<2 <2 <2	1.0±0	-	-	-	-
5	3 3 4	3.3±0.6	18%	-40% -40% -20%	-33%	60
10	7 5 3	5.0±2.0	40%	-30% -50% -70%	-50%	60
15	6 9 7	7.3±1.5	21%	-60% -40% -53%	-51%	30
15	5 8 6	6.3±1.5	24%	-67% -47% -60%	-58%	60
15	6 4 5	5.0±1.0	20%	-60% -73% -67%	-67%	120
25	12 11 11	11.3±0.6	5%	-52% -56% -56%	-55%	60
100	100 >100 100	100±0	-	-	-	60

^a For measured concentrations below the limit of detection, a mean value was calculated as half the limit of detection. For measured concentrations above the limit of detection, the upper limit of detection was used for to calculate the mean value.

ANDalyze correctly identified the blank samples of tap water as contained lead concentrations below the method limit of detection, 2 ppb (Table XX), and correctly identified standards of 100 ppb as being at the method upper limit of quantification, 100 ppb. However, for samples in the range of 5-25 ppb lead in tap water, ANDalyze results were all below the method detection limit, 2 ppb (Table XX). The impact of incubation time on method performance cannot be evaluated as all ANDalyze results were below the lower limit of detection.

5.3.3 ANOVA Results

In two-way ANOVA analysis for cadmium, water type and the interaction between the standard concentration and water type were not significant ($p=0.89$ and $p=0.57$, respectively), so in the final model, the interaction was omitted. Based on the results from the ANOVA analysis, ANDalyze performance was found to be statistically significantly different across the standard concentrations ($p<0.001$), with $R^2=0.84$. For the two-way ANOVA analysis for lead, ANDalyze performance for the measurement of lead was found to be statistically significantly different across the standard concentrations ($p<0.001$), water types ($p<0.001$), and that these two factors interacted ($p<0.001$). The R^2 was 0.99.

5.4 Discussion

The field-based fluorimetric biosensor system, ANDalyze, used to quantify known concentrations of cadmium and lead in two different water types showed inconsistent performance. Overall, the method was positively biased for cadmium (Table XVII and

TABLE XX
SUMMARY OF RESULTS FOR MEASURED LEAD FROM ANDALYZE BY TAP WATER

Lead Standard (ppb)	Result from ANDalyze (ppb)	Mean±SD ^a (ppb)	CV (%)	Percent Error	Mean Percent Error	Incubation Time (minutes)
0 (Blank)	<2 <2 <2	1.0±0	-	-	-	-
5	<2 <2 <2	1.0±0	-	-80%	-80%	60
10	<2 <2 <2	1.0±0	-	-90%	-90%	60
15	<2 <2 <2	1.0±0	-	-93%	-93%	30
15	<2 <2 <2	1.0±0	-	-93%	-93%	60
15	<2 <2 <2	1.0±0	-	-93%	-93%	120
25	<2 <2 <2	1.0±0	-	-96%	-96%	60
100	100 100 100	100±0	-	-	-	60

^a For measured concentrations below the limit of detection, a mean value was calculated as half the limit of detection. For measured concentrations above the limit of detection, the upper limit of detection was used for to calculate the mean value.

XVIII) and negative biased for lead in deionized water (Table XIX); in tap water, the method was unable to detect lead at concentrations between 5 and 25 ppb (Table XX). For cadmium, there were no samples measured by the ANDalyze method that were within $\pm 15\%$ of the standard concentration for both deionized water and tap water. For lead in deionized water, 44% (N=8) samples were correctly identified by ANDalyze within $\pm 15\%$ of the standard concentration; however, in tap water, no samples measured by the ANDalyze method that were within $\pm 15\%$ of the standard concentration.

Variability is inherent in this biosensor method due to the fact that no two biosensors are exactly the same (Lu, 2009). ANDalyze reports the accuracy of the biosensor system as $\pm 15\%$ (Dugan, 2015). To minimize variability and confirm with manufacturer specifications, we measured water quality parameters (pH, temperature, turbidity, conductivity), filtered tap water, controlled incubation time, and used plastic sample collection bottles (Van Geen et al., 2005). Many of these factors are known to affect the quantification of heavy metals analyzed by a variety of methods, including ANDalyze (Lu, 2009). However, field kits are designed to work under “real-world” conditions, not laboratory controlled environments with deionized water media only. It is necessary, from a practical standpoint, to verify performance of these kits in target media, such as tap water from different water systems and private wells. This give more confidence that field performance will match laboratory performance.

The presence of multiple metals in a water sample can interfere in the quantification of the target metal ion. ANDalyze tested and reported the levels for 15 chemicals that resulted in a $\pm 10\%$ change in the measured lead concentration. Six of

these metals (cadmium, copper, iron, lead, mercury, and zinc) and found that all were present below the interference level. We were unable to quantify the other nine chemicals: aluminum, ammonium, calcium, carbonate, chloride, magnesium, manganese, nitrate, phosphate, and sulfate; thus, these ions could potentially cause interference and inconsistency in the measured cadmium and lead concentrations.

Although the use of biosensors for the detection of heavy metals in water samples has been tested for several decades, published method performance studies in field settings are sparse (Bentley et al., 2001; Raja and Selvam, 2011; Rategarpanah et al., 2013; Guo et al., 2015; Li et al., 2015). A previous US EPA Environmental Technology Verification (ETV) report tested the ANDalyze system for the detection of lead in drinking water and concluded that method performance was subject to water quality characteristics dictated by environmental conditions and water quality characteristics artificially imparted on synthetic environmental or laboratory samples (Yates et al, 2013), similar to the results of this study. In addition, there was a major limitation with the ETV report: if on-site calibration failed after the second attempt or if acceptance criteria (quality control) was not met, samples were not analyzed, and the number of samples that did not meet this criteria was not reported (Yates et al, 2013). The number of samples unable to be analyzed is equally important to the number that were analyzed. The reported accuracy and precision in this report may not be accurate, as they are reflecting the total number of samples that met acceptance criteria rather than the total of samples attempted to be sampled.

5.4.1 Limitations

Future analyses would benefit from an increased number of standard concentrations for cadmium and lead, as well as other metals. The biosensor system has other heavy metal-specific sensors available, and the performance of those sensors should also be evaluated, as the CVs and percent errors of these other metal-specific sensors could be different from what was found in the cadmium and lead sensors in this research.

This study used a single source of purified deionized water and drinking water, and additional samples from different locations and other water sources, such as well water, would have enhanced the findings of this research. The quality of drinking water, including the presence of contaminants that interfere with sensor performance, may differ by infrastructure and water purification process on the detection of the sensor (Guidotti et al., 2007; Renner, 2009). However, similar results for other City of Chicago tap water sources would be expected, based on the annual drinking water quality report released by the city. The City of Chicago water quality for lead in drinking water is below the US EPA TT action level of 15 ppb lead (City of Chicago, 2015). Furthermore, although there was a single source of purified deionized water, this is not typical source of drinking water for the general population. The ANDalyze system was designed to be able to detect metals in drinking water sources and not just controlled laboratory samples, and as such, evaluations for field-based detection methods for drinking water should be designed for real-world conditions with multiple contaminants at trace (low-doses) concentrations.

Three different incubation times were considered, in order to determine if incubation of samples would impact the detection of cadmium or lead by the biosensor system. Baghel et al. found that the greater the concentration, the more rapid the detection time (Baghel et al., 2007). Van Geen et al. found that when using the Hach EZ arsenic test kit, increasing the reaction time greatly increased the measurement accuracy (Van Geen et al., 2005). Additionally, exploration of storage and additional incubation procedures are needed to determine those that will result in the highest degree of accuracy for different types of water samples. However, in this study, the results did not vary among incubation times by water type.

One specific limitation of the ANDalyze system is that the lower limit of detection of 0.1 ppm for the cadmium sensor is greater than the US EPA MCL for cadmium of 0.005 ppm (United States Environmental Protection Agency, 2014a). In terms of efficacy for field-based detection methods, the equipment should be able to detect concentrations of contaminants in real-world settings and at levels set by federal, state, and/or local drinking water regulations. The fact that the lower limit of detection for cadmium is 20 times the MCL, this sensor is not relevant for use to assess the quality of drinking water with respect to cadmium.

The ANDalyze system used for this analysis has since been replaced with a newer model. The manufacturer states that this new model “has twice the sensitivity” (ANDalyze, 2014). Because of this change, the results of this study could potentially be different and the detection of the cadmium and lead in both deionized water and tap water could be improved with the new model.

Despite these limitations, there are several strengths to this study. The standard concentrations for cadmium and lead were verified by an external laboratory with a gold-standard detection method. The analysis for the detection of lead drinking water was similar to a previous US EPA ETV report and the results from that report and from this research found that the ANDalyze system was subject to water quality characteristics dictated by environmental conditions and water quality characteristics of environmental or laboratory samples.

5.5 Conclusion

The biosensor system evaluated in this research, ANDalyze, has the potential to offer time-efficient and relatively inexpensive analysis of heavy metals, such as cadmium and lead, in drinking water. However, the ANDalyze fluorimeter (AND1000) did not prove to be a reliable device for measuring cadmium and lead in our hands. The ANDalyze system completely failed to detect lead in tap water samples at standard concentrations of 5-25 ppb. The analysis for the detection of lead in drinking water was similar to a previous US EPA ETV report and the results from that report and from this research found that accuracy of the ANDalyze system was affected by water quality characteristics dictated by environmental conditions. Further study is specifically warranted to explore whether the poor quantification of lead in tap water in this study is unique to the tap used or to drinking water in the City of Chicago. This study confirms that testing of analytical methods intended for analysis of environmental samples should go beyond laboratory conditions, and consider performance in environmental matrices

and field deployment. Future research is needed to study reliability and validity of this biosensor system for heavy metal mixtures.

6. RELIABILITY OF COMMERCIALLY AVAILABLE FIELD KITS FOR THE DETECTION OF LEAD IN DRINKING WATER WITH LEAD AND LEAD-MIXTURES

6.1 Introduction

Lead in drinking water remains a public health issue; however, the potential importance of different routes and sources of exposure for lead has been downplayed and questions remain over the impact of such chronic, low-dose exposure to lead (Renner, 2010; Ngueta et al., 2016). Exposure to lead in drinking water and the role that exposure plays in daily intake has been center of public health and media attention because of the issues in Flint, MI. Contaminated drinking water is the most common source of exposure to low levels of heavy metals, especially in the United States (US) (Agency for Toxic Substances and Disease Registry, 2007b). However, challenges remain in identifying and measuring contaminants in drinking water supplies at extremely low concentrations; therefore, new methods in exposure and risk analysis for human health are required (Villanueva et al., 2014). Furthermore, there are questions as to how chemical mixtures challenge the effectiveness of screening tools (Brack et al., 2015; Altenburger et al., 2015). Identification of specific contaminants and quantification of those detection limits become less accurate as the simultaneous presence of two or more substances may alter physicochemical properties of components such as solubility, which may affect bioavailability (Brack et al., 2015; Altenburger et al., 2015; Altenburger et al., 2003).

Commercially available field-based test kits designed for the detection of lead in drinking water allow consumers to test their drinking water at home, for a low cost. This is extremely poignant in light of recent events in Flint, MI. Flint was once a pivotal manufacturing center for General Motors (GM), but with the decline in auto

manufacturing and sales in recent years, the local economy has fallen into economic hardship (Sanburn, 2016). As a cost-saving measure, the city council voted to stop purchasing water from Detroit, and instead use a new regional pipeline, Karegnondi Water Authority that would, like Detroit, draw water directly from Lake Huron (Sanburn, 2016). However, a major problem arose from this decision: construction for the new pipeline had not even started before the City of Detroit retaliated and stopped selling water to Flint (Sanburn, 2016). The short-term solution while waiting for the new pipeline to be built was to have the City of Flint draw water from the Flint River (Bellinger, 2016; Sanburn, 2016). Unfortunately, corrosion control measures necessary to mitigate high levels of chlorine in the Flint River water and the addition of ferric chloride (used to reduce the formation of trihalomethanes from organic matter during treatment), were not applied (Bellinger, 2016; Sanburn, 2016). High corrosivity of water makes it easier for metals, like lead, to leach from the pipes and into the drinking water as it is transported to users (Bellinger, 2016). Months after residents voiced their concerns over the quality of their drinking water, initial testing found six of nine city wards, water in over 20% of homes tested had lead concentrations greater than 15 ppb, the US EPA's TT action level; some samples were in excess of 100 ppb (Bellinger, 2015; United States Environmental Protection Agency, 2014d).

As awareness among the general public of the hazards of lead piping and decaying infrastructure across the US increases, so does the importance of assessing that field-based detection kits are providing accurate results to the general public. Current detection methods for testing heavy metals in drinking water are typically laboratory-based, time intensive, costly, not always metal specific, not readily available

for field use, and require specialized training (Raja and Selvam, 2011; Rategarpanah et al., 2013). Thus, there is a need for sensitive, effective, and cost-efficient methods capable of accurately detecting heavy metals such as lead in drinking water supplies in the field. Commercially available test kits are one option to fulfill this need.

There have been a number of studies in the literature which evaluate the validity and reliability of field-based methods for detection of heavy metals, but many of them focus on the detection of a single heavy metal, particularly arsenic, and are tested in known areas with elevated concentrations in drinking water (Van Geen et al., 2005; Jakariya et al., 2007; Baghel et al., 2007). In the US, however, consumers are interested in quantifying or detecting the presence of one or more heavy metals at levels near regulatory limits, which are low relative to the levels tested in previous studies of field-based methods.

The primary objective of this research is to evaluate the performance of three field-based test kits marketed for use by the general public to evaluate lead in residential drinking water. These kits are low cost (ranging from \$12.99 to \$49.95) and are available online and in major retailers. The field-based kits were evaluated for their accuracy and precision, using laboratory-based approach involving spiked samples of lead alone, and in mixtures with other metals, in purified deionized bottled water and drinking water obtained from a tap.

6.2 Methods

6.2.1 Field Kits

The three kits evaluated were: First Alert Drinking Water Test Kit (First Alert; First Alert Trust, BRK Brands, Inc.; Aurora, IL), WaterSafe Water Test Kit for Lead (WaterSafe; DiscoverTesting.com, Silver Lake Research Corporation; Monrovia, CA), and Pro-Lab Lead in Water Test Kit (Pro-Lab; Professional Laboratories; Weston, FL) (Appendix B). Both First Alert and WaterSafe are home-based tests that use colorimetric test strips to report the presence of lead above a certain concentration (First Alert Trust, 2005; DiscoveringTesting.com, 2015). In contrast, Pro-Lab is mail-away test kit, meaning that the consumer collects the water and then mail it back to the company for analysis.

6.2.2 Water Sources

Two water sources were used: purified, deionized bottled water (Thermo-Fisher Scientific, Hanover Park, IL) (deionized water) and environmental drinking water (tap water) drawn from a tap supplied by the City of Chicago. The deionized water was stored at room temperature, at approximately 23.5°C (74.3°F). The manufacturer's specifications for tap water collection and temperature differed among the field kits. And tap water was collected from the same tap following the manufacturer's instructions. For the First Alert and WaterSafe kits, prior to testing, the cold tap was run for 15-20 seconds (First Alert Trust, 2005; DiscoverTesting.com, 2011). No temperature specifications were provided, so analysis was performed once the sample reached room temperature, approximately 23.5°C (74.3°F). For the Pro-Lab kit, the cold water

tap was turned on to a slow trickle and run until a plastic one-liter container was full (Pro-Lab, 2013).

6.2.3 Procedure for Expected Concentrations of Lead in Water Samples

All the standard used for spiking were purchased in a 2% nitric acid (HNO_3) solution from SPEX CertiPrep, Custom Assurance Standard (Metuchen, NJ). A stock solution was used to make serial dilutions for spiking. Serial dilutions were made from deionized and tap water, in certified RNAase-/DNAase-free, heavy metal free color concentrate, medical grade polypropylene, nonpyrogenic centrifuge tubes (Corning, Corning, NY). Serial dilutions were made once the water reached room temperature (within three hours of sample collection) and used with two hours. For deionized water, there were four lead standards: 5 ppb, 10 ppb, 15 ppb, and 25 ppb. For tap water, there were seven lead standards: 5 ppb, 10 ppb, 13 ppb, 15 ppb, 17 ppb, 20 ppb and 25 ppb. These standard concentrations were determined based on US EPA's TT action level for lead and the detection limit of the kit (United States Environmental Protection Agency, 2014a). Three lead-copper mixtures were made: each contained 15 ppb lead and 50 ppb, 1300 ppb, or 2000 ppb copper. The first two levels of copper correspond to levels in the City of Chicago tap water and the US EPA treatment technique action level, respectively (City of Chicago, 2015; United States Environmental Protection Agency, 2014a). Three lead-iron mixtures were made: each contained 15 ppb lead and 100 ppb, 300 ppb, or 1000 ppb iron. Iron at 300 ppb is the US EPA Secondary Drinking Water Standard at which there is visible discoloration of water (United States Environmental Protection Agency, 2016a). In addition to the standard concentrations,

blank (non-spiked) water samples of both deionized water and tap water were tested in order to establish baseline levels of lead in the water samples. Summary of the standard concentrations can be seen in Tables V-IX in [Section 3.4.4.3 Sampling Preparation](#).

6.2.4 Procedure

The First Alert kit was used in accordance with manufacturer's instruction (First Alert Trust, 2005). Briefly, using the provided sampling equipment, two full droppers of water were placed into the test vial; the vial was gently mixed (swirled) for several seconds then placed onto a flat surface. The test strip for lead was then inserted into the vial for ten minutes. Based on the package insert from the manufacturer, a negative result is indicated when "the bottom line (next to the number 1) is darker than the top line (next to the number 2)" or "if you only see one line next the number 1". A positive result is indicated when "the top line (next to the number 2) is darker than the bottom line (next to the number 1), or lines are equally dark". The manufacturer notes that "if a test strip shows a positive result, your water sample may contain lead at a toxic level". The test result is not valid "if no lines appear, or both lines are very light". All samples were tested in triplicates with negative (blank) controls and positive (100 ppb lead) controls. The First Alert kit has a detection limit of 15 ppb lead.

The WaterSafe kit was used in accordance with the manufacturer's instructions (DiscoverTesting.com, 2011). Briefly, using the provided test vial and dropper pipette, one pipette-full of the sample was placed into the test vial and the test vial was swirled gently and then placed on a flat surface. The WaterSafe test strip was then inserted

into the test vial, arrow pointed down, for 10 minutes. The reading and interpretation of the WaterSafe test strip is identical to that of the First Alert test strip. All samples were tested only in tap water, in triplicates with negative (blank) controls and positive (100 ppb lead) controls. The WaterSafe kit has a detection limit of 15 ppb lead.

The Pro-Lab test kit was used in accordance with the manufacturer's instructions, which included specific directions for how to collect the water sample (Pro-Lab, 2013). Briefly, once tap water was collected in a plastic container and stirred with a non-metal spoon. The provided plastic test tube was filled with tap water immediately after the water was stirred. The remaining tap water was divided into plastic conical test tubes for serial dilutions. Once serial dilutions were made, the tap water sample with the desired standard (lead, lead-copper, lead-iron, or lead-copper-iron) was poured into the provided plastic test tube. The water sample was mailed within four hours of sample collection, well within the manufacturer's specification of 24 hours of the sample collection. A week after the water samples were mailed, a chain of custody email was received, followed by the results within two weeks. According to Pro-Lab, the laboratory analysis used to analyze lead in water samples is inductively couple plasma atomic emission spectrometry (ICP-AES), following EPA Method 200.7 (Pro-Lab, 2016). The lower limit of detection for ICP-AES is 0.01 ppm (United States Environmental Protection Agency, 2001). Only single samples were sent to Pro-Lab for analysis.

6.2.5 Quality Assurance and Quality Control

To ensure quality assurance and quality control, all water samples (deionized and tap) were tested with First Alert in triplicate and tap water samples were tested with

WaterSafe in triplicate, in sequence with positive and negative controls. Single water samples were also sent to the University of Chicago, Department of Chemistry, Mass Spectrometry Core Facility, an external lab, for ICP-MS analysis. The ICP-MS limit of detection for lead was 0.1 ppb, whereas for common elements like zinc and iron, the limit of detection is 1 ppb. A subset of samples, the four standard lead concentrations in deionized water and in tap water, and five mixtures of 15 ppb of lead with known concentrations of copper and/or iron, along with one sample of blank of deionized water and tap water were sent for analysis. Samples were prepared in 2% nitric acid (HNO_3) solution with 18 mega-Ohm ($\text{M}\Omega$) doubly-deionized water. Water samples were shipped to the University of Chicago within five hours of sample preparation, received the following day and analyzed the following business day.

All First Alert and Pro-Lab kits were purchased in two batches, February and May 2016, directly from the manufacturer. The first set of WaterSafe kits were purchased from a commercial online retailer (February 2016) and a second set of kit was purchased (May 2016), directly from the manufacturer. The expiration date for the First Alert kits was February 2017. The expiration date for the first set of WaterSafe kits was December 2016 and April 2018 for the second set of kits.

Lastly, water quality parameters of pH, hardness, chlorine, nitrates, bacteria, turbidity and conductivity were measured. The First Alert field kit provided test strips for pH, hardness, chlorine, nitrates, and pesticides, with test vial and instructions for bacteria.

6.2.6 Statistical Analysis

Since First Alert and WaterSafe yield binary response, we tabulated true and false positives and true and false negatives, and used these data to calculate sensitivity, specificity, true positive rate, false positive rate, precisions and accuracy, including negative (blanks) and positive controls. A true positive (TP) was when the test strip indicated positive result for a sample with a standard concentration that was at or above the manufacturer's detection limit of 15 ppb lead, whereas true negative (TN) was when the test strip indicated a negative result for a sample with a standard concentration that was below 15 ppb lead. A false positive (FP) was when the test strip indicated a positive results for a sample with a standard concentration that was below the manufacturer's detection limit of 15 ppb lead, and a false negative (FN) was when the test strip indicated a negative result for a sample with a standard concentration that was above 15 ppb lead. Test strips from the First Alert kits and the WaterSafe kits that resulted in an "error" reading were not included in the final performance measure calculations.

Since an actual concentration was provided from the Pro-Lab kit, a two-way (factor) ANOVA without replication analysis was conducted to determine if the measured concentration was different from the expected concentration, by water type, and the interaction of these two factors. The dependent variable was the measured concentration by Pro-Lab, with the independent variables the standard concentrations (DF of seven), water type (DF of one), and the interaction between the standard concentrations water type (DF of two). Additionally, a coefficient of determination, R^2 , was calculated.

The concordance correlation coefficient was calculated to evaluate inter-rater reliability for a subset of 14 samples of lead standards in tap water for both the First Alert and WaterSafe test strips. Two scientists categorized the results of the test strips into three categories for each test strip - negative, positive, and error. SAS software (v 9.4, Cary, NC) was used for all statistical analyses.

6.3 Results

All water quality parameter measures were in deionized and tap water samples prior to serial dilutions. Samples had a temperature of approximately 20.8°C, with a pH of 6 for the deionized water and a pH of 7 for tap water. For tap water samples, the conductivity was 314 micro-Siemens (μS) and the turbidity was 0.3 Nephelometric Turbidity Units (NTU). The hardness of the tap water was between 50 and 120 ppm, meaning that the tap water has moderate hardness. Total chlorine was 0 ppm, below the 4 ppm maximum residual disinfectant level set by US EPA (United States Environmental Protection Agency, 2014a). Total nitrate/nitrite was 0 ppm, below the US EPA's maximum contaminant level of 10 ppm, while nitrite was 0 ppm, also below US EPA's maximum contaminant level of 1 ppm (United States Environmental Protection Agency, 2014a). The pesticides test strip obtained a negative result. Lastly, the bacteria test was negative, meaning that there was no detectable bacteria in the tap water. A subset of 14 samples in tap water for both First Alert and WaterSafe were reviewed by two reviewers. For First Alert, the concordance correlation coefficient was 0.81 (95% CI: 0.52, 0.93). For WaterSafe, the concordance correlation coefficient was 1.00. The ICP-MS results confirmed that the standards were made as expected for the

standard lead concentrations in both deionized water and tap water, but the ICP-MS results for the standard concentrations for lead-mixtures were below what was expected (Table XXI).

TABLE XXI
ICP-MS ANALYSIS RESULTS FOR LEAD AND LEAD-MIXTURES

Lead Standard (ppb)	Copper Standard (ppb)	Iron Standard (ppb)	Result for Lead (ppb)	Result for Copper (ppb)	Result for Iron (ppb)
0 (Deionized Water Blank)	-	-	<0.000	<0.000	<0.000
0 (Tap Water Blank)	-	-	2.3	209.8	15
5	-	-	6.4	<0.000	<0.000
10	-	-	12.6	<0.000	<0.000
15	-	-	19.0	<0.000	<0.000
25	-	-	30.5	<0.000	<0.000
15	1300	-	8.3	721.0	0.02
15	-	300	9.1	0.02	166.7
15	1300	300	6.7	465.5	108.1
15	1300	1000	6.3	483.8	352.4

The results from the First Alert test kits for standard lead concentrations in deionized water are shown in Table XXII. In deionized water, the blank (negative control), 5 ppb and 10 ppb lead only standard samples were all correctly identified as below the method threshold, 15 ppb, and were classified as TNs. All 25 ppb and 100 ppb lead only standard samples were correctly identified as above the method threshold, 15 ppb, and were classified as TPs. The standard lead concentrations for 15 ppb resulted in errors. For the lead only standard in deionized water, the FP and FN rates could not be calculated because there were FPs and FNs, respectively. The

sensitivity was 100% and the specificity was 100%, with an accuracy of 100% and precision of 100%.

TABLE XXII
FIRST ALERT RESULTS FOR LEAD IN DEIONIZED WATER

Lead Standard (ppb)	Result
0 (Blank)	TN
	TN
	TN
5	TN
	TN
	TN
10	TN
	TN
	TN
15	Error
	Error
	Error
25	TP
	TP
	TP
100 (Positive Control)	TP
	TP
	TP

The performance of First Alert test kits for 15 ppb lead concentrations in mixtures in deionized water resulted in errors in two of 27 samples (7%), but only one samples, with 100 ppb iron was TP; all others were FNs (Table XXIII). For the lead mixtures in deionized water, the FN rate was 86%, sensitivity was 14%, accuracy was 23%, and the specificity and precision were both 100%.

TABLE XXIII
FIRST ALERT RESULTS FOR LEAD MIXTURES IN DEIONIZED WATER

Lead Standard (ppb)	Copper Standard (ppb)	Iron Standard (ppb)	Result
15 ppb	50 ppb	-	FN
			FN
			FN
15 ppb	1300 ppb	-	FN
			FN
			FN
15 ppb	2000 ppb	-	FN
			FN
			FN
15 ppb	-	100 ppb	Error
			Error
			TP
15 ppb	-	300 ppb	FN
			FN
			FN
15 ppb	-	1000 ppb	FN
			FN
			FN
15 ppb	1300 ppb	100 ppb	FN
			FN
			FN
15 ppb	1300 ppb	300 ppb	FN
			FN
			FN
15 ppb	1300 ppb	1000 ppb	FN
			FN
			FN

The results for the First Alert Kits for lead in tap water are displayed in Table XXIV. Compared to deionized water, the results for the lead only standard in tap water are more variable, with 30% of samples resulting in errors and 23% of samples resulting in FNs. For lead standards, the FP rate was unable to be calculated because there were no FPs, but the FN rate was 64%, the sensitivity was 29%, specificity was 100%, accuracy was 67%, and precision was 100%.

The results for the First Alert for 15 ppb lead in mixtures in tap water were all FN (Table XXV). In mixtures, the performance decreased with a FN rate of 90%, sensitivity was 10%, specificity was 100%, accuracy was 20%, and precision was 100%. As there were no FPs, the FP rate could not be calculated.

The results from the WaterSafe kits are shown in Table XXVI. Among the lead only samples, errors occurred for all samples with 13 ppb and 15 ppb lead, and for two of three samples with 20 ppb lead. Errors occurred in 11 of 30 (37%) of samples. TPs were observed for 25 ppb and 100 ppb lead only standard samples, while TNs were observed for samples with ≤ 10 ppb lead. For the lead only standards, the FN rate was 40%, sensitivity was 60%, specificity was 100%, accuracy was 79%, and precision was 100%. When lead was present at 15 ppb in mixtures, the WaterSafe kit did not return any positive results; all samples were FNs. For the mixtures of lead standards, the FN rate was 75%, sensitivity was 25%, specificity was 100%, accuracy was 40%, and precision was 100%.

TABLE XXIV
FIRST ALERT RESULTS FOR LEAD IN TAP WATER

Lead Standard (ppb)	Result
0 (Blank)	TN
	TN
	TN
5	TN
	TN
	TN
10	TN
	TN
	TN
13	TN
	Error
	Error
15	Error
	Error
	Error
15	Error
	Error
	Error
17	FN
	FN
	FN
20	FN
	FN
	Error
25	FN
	TP
	FN
100 (Positive Control)	TP
	TP
	TP

TABLE XXV
FIRST ALERT RESULTS FOR LEAD MIXTURES IN TAP WATER

Lead Standard (ppb)	Copper Standard (ppb)	Iron Standard (ppb)	Result
15	50	-	FN
			FN
			FN
15	1300	-	FN
			FN
			FN
15	2000	-	FN
			FN
			FN
15	-	100	FN
			FN
			FN
15	-	300	FN
			FN
			FN
15	-	1000	FN
			FN
			FN
15	1300	100	FN
			FN
			FN
15	1300	300	FN
			FN
			FN
15	1300	1000	FN
			FN
			FN

TABLE XXVI
WATERSAFE RESULTS FOR LEAD AND LEAD MIXTURES IN TAP WATER

Lead Standard (ppb)	Copper Standard (ppb)	Iron Standard (ppb)	Results
0 (Blank)	-	-	TN
			TN
			TN
5	-	-	TN
			TN
			TN
10	-	-	TN
			TN
			TN
13	-	-	Error
			Error
			Error
15	-	-	Error
			Error
			Error
15	-	-	Error
			Error
			Error
17	-	-	FN
			FN
			FN
20	-	-	FN
			Error
			Error
25	-	-	TP
			TP
			TP
100 (Positive Control)	-	-	TP
			TP
			TP
15	1300	-	FN
			FN
			FN
15	-	300	FN
			FN
			FN
15	1300	300	FN
			FN
			FN

The results from the Pro-Lab kits are shown in Table XXVII. The measured concentrations of lead were provided by Pro-Lab in a one-page certificate of water analysis document (Appendix C). The report lists the analyte tested (in this case lead), the result, the acceptable range, and the determination (whether the result is above or below of the TT action level of 15 ppb). Of the 27 samples sent to Pro-Lab, results were not obtained for four samples (17%). For the Pro-Lab measured lead concentrations in tap water, 64% of were outside a $\pm 15\%$ accuracy range.

In addition, two sample results in excess of the TT action level for lead were incorrectly identified by Pro-Lab as “pass”. The two-way (factor) ANOVA analysis found that there were statistically significant differences between the Pro-Lab measured concentrations and the standard concentrations ($p=0.05$), water type ($p=0.002$), and the interaction of these two variables ($p=0.01$), with and $R^2=0.89$.

6.4 Discussion

The three field-based kits evaluated for the detection of lead in drinking water varied in performance, among the kits, when lead was present alone or in mixtures, and when lead was in deionized or tap water. In tap water, the First Alert and WaterSafe kits resulted in errors as the lead only standard concentrations were 13 ppb and 15 ppb, where 15 ppb is the method detection limit and should provide a positive result. Overall, for all three test kits, the performance for lead mixtures in tap water were highly variable, with the First Alert and WaterSafe kits resulting in mostly all FNs.

TABLE XXVII
PRO LAB RESULTS FOR LEAD AND LEAD MIXTURES IN DEIONIZED WATER
AND TAP WATER

Water Type	Lead Standard (ppb)	Copper Standard (ppb)	Iron Standard (ppb)	Blank-Corrected Lead Concentrations from Pro-Lab (ppb)
Deionized Water	0 (Blank)	-	-	ND ^a
	5	-	-	5
	10	-	-	12
	15	-	-	NA
	25	-	-	2
Tap Water	0 (Blank)	-	-	2
	5	-	-	11
	10	-	-	16
	13	-	-	5
	15	-	-	20 ^b
	15	-	-	NA
	17	-	-	17
	20	-	-	20
	25	-	-	35 ^b
	15	50	-	13
	15	1300	-	14
	15	2000	-	NA ^c
	15	-	100	NA ^c
	15	-	300	13
	15	-	1000	11
	15	1300	100	11
	15	1300	300	9
	15	1300	1000	12

^a Not detected (ND) at or above the limit of detection

^b Results are above the US EPA's action level for lead, but were originally marked "Pass" by Pro-Lab

^c NA indicates that samples were sent, but no analysis/results were provided by Pro-Lab

In general, the way in which water is collected can impact the results for the detection of lead, which ultimately is an issue for exposure assessment (Renner, 2010). In order to minimize the impact on water collection methods on the method performance, the manufacturer's instructions were followed, when available. The differences in how water is collected and sampled have been at the center of debate for a number of years (Renner, 2010). One of the sampling parameters that has been questioned is the water temperature (Renner, 2010). Cold water is typically sampled, as was specified by the Pro-Lab test kit (Pro-Lab, 2013; Renner, 2010). However, the use of cold water samples may not be accurate collection technique for the assessment of human exposures as more lead may be released into water from hot water (Renner, 2010). Another issue is the collection tubes/containers. Like listed in the Pro-Lab instructions, the water was first collected in one container and then poured into another (Pro-Lab, 2013; Renner, 2010). However, during this process, heavy lead particles may sink to the bottom of the container and may not be present in the sample (Renner, 2010). In order to correct this potential issue, the water collected for the Pro-Lab kits was stirred (as directed by the package insert), while the samples for the First Alert and WaterSafe kits was gently stirred before placing the sample in the vials for testing. Furthermore, the stream/speed of the water from the tap may also cause differences in measured lead concentrations, as more lead may be released with a heavier flow of water (Renner, 2010). The Pro-Lab kit specifically stated for the cold water to be turned on to a slow trickle, and for consistency, the water used in the First Alert and WaterSafe kits also was collected from a slow trickle, after a slow trickle used to flush the cold tap (Pro-Lab, 2013).

The Pro-Lab test kits are a mail-away kit, and we did not receive results for all samples submitted. In total, 27 samples were mailed to Pro-Lab for analysis of lead. However, confirmation and results for only 23 samples were received from Pro-Lab, two samples were never received, and two samples were received for radon testing. Attempts to contact Pro-Lab about how two samples were labeled for radon testing and two samples were not received altogether went unanswered.

In addition, two sample results received from Pro-Lab were reported to be above the TT action level of 15 ppb – 18 ppb and 33 ppb (Appendix C), but the determination was “Pass”. Pro-Lab was contacted with this concerning issue, and the certificate of water analysis were reissued with “Fail” determinations. Although the results were reissued with the corrected determination, this even left us concerned about the quality control processes in place at the company. Altogether, these observations have generated concern among the research team that consumers will be disappointed, confused, and frustrated by the services of Pro-Lab. Inaccurate results can lead to high anxiety about potential adverse health outcomes, or false security, and failure to return results is clearly a poor business practice.

The results for the statistical measures of performance evaluating the three field-based test kits can be misleading due to frequency of errors (excluded from analysis) and absence of some outcomes (TP, FP, TN, and FN), making it appear that the kits perform better than they actually do. For example, there were no FPs for the First Alert and WaterSafe kits, so both the specificity and precision were 100%, and a false positive rate could not be calculated. The absence of FPs in the measured lead concentrations could mean that the kits can accurately detect samples without or lower

detectable concentrations of lead; however, based on the results from this research, more samples would be need to be tested to draw further conclusions. This needs to be considered when evaluating the performance and results. The number of samples needed could be calculated using sensitivity and specificity to generate a receiving operating characteristic (ROC) curve, and then calculating the area under the curve (AUC).

In the presence of copper and/or iron, the concentration of lead was masked; in the Pro-Lab results, the lead concentration was lower than expected and in the WaterSafe and First Alert kits, lead was not detected. Both the ICP-MS analysis and the Pro-Lab results measured similar concentrations of lead when it was in mixture with copper and/or iron, meaning that the two methods have similar accuracy in detecting lead in a mixture. For the standard with lead only at 15 ppb, the Pro-Lab analysis and the ICP-MS measured the concentration as 20 ppb and 19 ppb, respectively; however, when in mixture with copper and/or iron, the Pro-Lab and ICP-MS results ranged from as 9 ppb to 14 ppb. It is unlikely that this difference reflects an error in the standard solution, as all standards with 15 ppb lead were from the same serial dilution sample. The result of copper and/or ion acting as. Whether this antagonism effect between lead and copper and/or iron is functional (counterbalancing of the toxic effects), chemical (copper, iron, and/or another contaminant react with lead to reduce toxicity), receptor (lead and another contaminant are bound to a receptor, and the concentration of lead is lessened) or dispositional (the disposition of lead is altered, i.e. no longer free lead, so that the concentration is diminished) is unknown, but it warrants further analysis, as this means measurements of lead in drinking water are likely underestimated.

There are limited studies on the effects of heavy metal mixtures in drinking water and the effect on detection; however, there are numerous studies that show explain observed correlations between lead and iron in drinking water through adsorption mechanisms using synthesized iron oxides (Trueman and Gagnon, 2016). As a way to remove heavy metals from drinking water, nanosized metal oxides have been used experimentally (Hua et al., 2012). In particular, iron-based metal oxides were shown to bind with target metals, like lead and copper, for sorption and removal (Hua et al., 2012). In addition, high iron often accompanies elevated lead in drinking water (Trueman and Gagnon, 2016). The propensity for lead to adsorb onto iron surfaces is due to high solid-water partition coefficient (Trueman and Gagnon, 2016). Removal of heavy metals from drinking water is ongoing exploration into technologies and methodologies that could aid in this process. Studies have shown that the ability for lead to be bound to iron is an efficient strategy for removal (Barakat, 2011; Fu and Wang, 2011; Trueman and Gagnon, 2016), however, these studies also show that this relationship could have the potential to interfere with detection of lead in drinking water.

The three kits evaluated here were specifically chosen because of their availability and affordability. The test kits ranged in price from (approximately) \$13 to \$50, with estimated annual sales for the companies as follows: \$662 million for First Alert, \$2.27 million for WaterSafe's parent company, and \$78 million for Pro-Lab (Hoover's Inc., 2016a; 2016b, 2016c). These at-home test kits provide empowerment to the consumer, especially in situations like that of Flint, MI. The availability of these products offer consumers that ability to test their own drinking water and get their own results, amidst conflicting reports on the quality their drinking water that cause distrust

of government agencies in charge of water testing and monitoring. However, the results here indicate that there is high variability among test performance and the interpretation of results, which can only cause further frustration and confusion. When buying these products, consumers have the expectation that these multimillion dollar companies have properly tested their products to ensure performance accuracy and reliable results, but the results of this study question the accuracy of detection limits of test strips and within lead mixtures.

6.4.1 Limitations

The process in which water is collection can cause differences in measured concentrations. The use of hot or cold water may draw water through the tap that have different levels of lead. As the Pro-Lab kit specifically specified cold water to be used, the same process and temperature was used for the First Alert and WaterSafe kits for consistency among the samples. Another collection parameter that could be tested is the difference in the stream for collection, meaning if a heavier stream of water results in different measured concentrations than a slow trickle. Therefore, having more samples which tested these differences would provide a more comprehensive understanding of how collection techniques effect measured concentrations of lead in a sample and which technique could potentially provide a more representative sample estimate of lead from drinking water sources.

In addition to collection techniques, this research would have been enhanced by the use of drinking water from different locations in Chicago and from other cities and states, as well as from other water sources, such as well. Older and/or poorly

maintained infrastructure could potentially cause increased levels of contaminants (lead and other heavy metals) in the drinking water supply, which in turn could interfere with the field kit's precision to accurately detect heavy metals (Guidotti et al., 2007; Renner, 2009). In addition, water purification processes at water treatment plants and in the home can impact the contaminants (biological and chemical) in the drinking water supply, which could alter the measured concentrations of lead by the field kits (Guidotti et al., 2007; Renner, 2009).

Even with these limitations, this research provides insight into the performance of commercial available field kits in the context of drinking water with heavy metals at public health relevant concentration (the US EPA's MCLs and TT action levels). Furthermore, the evaluation of the finding that lead measurements were negatively biased in the presence of copper and iron indicate the need for further evaluation on the detection and quantification of a single analyte.

6.5 Conclusion

With the increasing attention on lead in drinking water supplies, having a reliable field-based method for detecting all heavy metals in drinking water that can be used by community members to test their water supply has public health value. The current literature evaluating field-based tests for detecting heavy metals in drinking water is largely focused on the detection of arsenic, not lead. The three field-based kits evaluated in this research have the potential to offer time-efficient, low-cost access to information about lead levels in drinking water for the public. However, although the three field-based kits had higher performance accuracy for detecting lead when it is the

sole contaminant in tap water, they fail to reliably and accurately detect lead when it is present in a mixture with copper and/or iron. Further research is needed to better understand why these methods were adversely impacted by the presence of mixtures. In the wake of Flint, MI, the awareness about lead in drinking water has been heightened. Consumers who are concerned about their drinking water can go buy one of these relatively inexpensive “at-home” kits to provide a sense of security. However, all three test kits had their issues; the interpretation of the test strips from First Alert and WaterSafe are dependent on subjectivity and there were numerous errors as the standard concentration approached the detection limit, while Pro-Lab had poor reliability with receiving and processing samples, and with quality control of the results. As indicated by the results of this study, the presence of other heavy metals may cause interference with accurately detecting lead in drinking water, providing consumers with a false sense of security, with larger public health implications overall.

7. DISCUSSION

This dissertation focused on three main research aims: (1) examine and characterize the number of cases of heavy metal poisonings in Illinois from 2010 through 2013 using the Illinois Hospital Discharge database; (2) evaluate the validity and reliability of a newly developed field-based biosensor methodology for the detection of cadmium and lead in drinking water across known concentrations; and (3) evaluate the reliability of three commercially available, mass marketed field-based kits for the detection of lead and lead-mixtures across known concentrations.

7.1 **Hospitalizations from Heavy Metal Poisoning Cases in Illinois, 2010-2013**

From 2010 through 2013, there was a total of 437 outpatient and 302 inpatient heavy metal poisoning cases treated in Illinois hospitals. The age-adjusted incidence rates for heavy metal poisonings showed a statistically significant increasing trend between 2010 and 2013 ($p < 0.05$), but because of the short-term period of follow-up it is unclear if this is capturing a long-term change resulting from increasing exposure or improved screening, or whether it is just a short-term aberration. The cumulative hospital charges for outpatients was \$1,566,506 and \$10,620,573 for inpatients over the four year period. Children (18 years or younger) compromised 30.6% of cases, but they were predominately lead exposures. Overall, there were no antimony cases and for inpatient cases, there were no cadmium inpatient poisoning cases. There were five cases of beryllium poisonings, which are expected to be exclusively occupational exposure cases, as beryllium is used in select industrial processes. Beryllium is a sensitizing agent, so occupational exposure limits are relatively low, and very low levels

(0.03 ng/m³ in air and 0.002 ppb in water) are found in the environment (Agency for Toxic Substances and Disease Registry, 2002). Only two of the five cases, however, occurred in patients over the age of 18. It is unclear if the child exposures to beryllium resulted from carry home exposures by their parents who work with beryllium or were the result of environmental sources.

An interesting finding was that for both outpatients and inpatients, there were no reported poisoning cases for chromium in 2010 or 2011, but in 2012 and 2013 there was a sudden increase in the number of cases; for outpatients 50 cases in 2012 and 65 in 2013, and for inpatients, 10 cases in 2012 and 8 in 2013. The reason for this sudden increase is unknown. However, the increase for some of the cases could potentially be attributed to increased public awareness following news reports in August 2011 about elevated levels of hexavalent chromium in drinking water in Chicago, which may have translated to an increase in testing by medical professionals within hospitals (Hawthorne, 2011; Rodriguez, 2011). It should be noted that the highest proportion of chromium cases in 2012 and 2013 were in Peoria County (N=16, 12%), a county in central Illinois and relatively far from Chicago. In addition, the patients with chromium exposures were substantially older than the other heavy metal patients, average age over 60 years, and all cases of chromium exposures occurred concurrently with exposures to mercury or unspecified other heavy metals. This trend in increasing chromium poisoning cases in Illinois was not seen in the national AAPCC annual reports for 2010 through 2013 and this trend cannot be verified nationally, as NHANES does not test for chromium in their survey (Bronstein et al., 2010; Bronstein et al., 2011; Mowry et al., 2012; Mowry et al. 2013). Further investigation to identify the point source

for these exposures is warranted based on their apparent specificity in time, space, and co-exposures among elderly patients.

Although there was a statistically significant difference between outpatient and inpatient cases based on race/ethnicity ($p < 0.05$) and discharge status ($p < 0.05$), 25% of the race/ethnicity categories and 69% of the categories for discharge status had a small sample size ($N < 5$), so the Chi-squared test may not be valid. There was not a statistically significant difference between outpatient and inpatient cases based on payor type ($p > 0.05$), but 54% of payor categories had small sample size ($N < 5$), so the Chi-squared test may not be valid.

There were more cases identified as general lead poisoning cases rather than being identified as having exposures from paint contaminated with lead, which has a different ICD-9-CM code. All lead paint poisonings occurred in children under 18 years of age, and nearly all the lead poisonings that did not identify paint as the main source of exposure occurred in children as well (79.0%; 135 of 171 cases). Additionally, for both outpatients and inpatients, all heavy metal categories had two or more exposures, with the exception of lead. In Illinois, of the children that were tested, there was a total of 1,701 (1.1%) confirmed cases of children who had blood lead levels greater than 10 $\mu\text{g/dL}$ in 2013 (Centers for Disease Control and Prevention, 2016a).

7.1.1 Comparison to National Survey Data

As stated previously, there are currently two primary surveillance tools that can be used to examine exposures to heavy metals in the general population – NPDS and NHANES. Between 2010 and 2013, the AAPCC recorded the following number of

single exposures to heavy metals in Illinois: 63 arsenic, 9 cadmium, 330 lead, 3 manganese, 10 mercury, 125 elemental mercury, 275 other, and 3 unknown (National Poison Data System, 2016).

Because nearly all the lead exposure cases occurred in children, it is unlikely to be of an occupational source. With the recurring issues of environmental contamination and the susceptibility of children to lead's adverse health effects it is important to invest in more robust surveillance of lead poisoning in children. Many states only require a single lead test for children before entering kindergarten, at which point it may already be too late. The robust literature demonstrates the impact adverse cognitive and behavioral effects at low doses at earlier ages, with lead having a slow clearance from the body, with half-life of 30 days in blood, but 27 years in bone (Bellinger et al., 1991; Canfield et al., 2003; Evens et al., 2015; Agency for Toxic Substances and Disease Registry, 2007b). However, despite these adverse effects, there are currently only 29 funded states for child lead surveillance programs and limited funding for Adult Blood Lead Epidemiology and Surveillance (ABLES) (Centers for Disease Control and Prevention, 2016b; Centers for Disease Control and Prevention, 2015b).

Poison center data provides comprehensive information of both aggregate and substance specific exposures, in addition to dose, severity, and outcomes; however, poison center data comprises primarily of minor exposures not involving adverse health effects that require treatment in health care facilities. In contrast, national health surveys capture patients treated in outpatient and inpatient settings (Web-based Injury Statistics and Query and Reporting System, 2012; National Health Interview Survey, 2012; National Hospital Discharge Survey, 2011; Ambulatory Health Care Data, 2009).

Without having the biological measure from the hospital discharge data, the comparisons to NHANES data is more complex, as making determination of what is considered a poisoning directly from the NHANES data may create misclassification of poisoning cases, since NHANES only reports the measured level for heavy metals in the body not if the person suffered adverse health effects from these exposures. Thus, utilizing hospital discharge data could help to fill the gap of national surveillance data.

7.1.2 Limitations

The determination of a heavy metal poisoning was based on ICD-9-CM codes. Since ICD-9-CM codes are for the purposes of coding/billing, there is no information provided on dose; thus, quantity of the heavy metal, laboratory confirmation of the presence of heavy metal(s), timing of the initial exposure, the frequency of exposure, and timing of laboratory findings is not available. Additionally, the use of ICD-9-CM codes may be missing heavy metal poisoning cases because the codes may reflect the clinical presentation and outcome, rather than the underlying causative agent. Because of this, it is difficult to determine how exposure to heavy metal(s) played a role in the diagnosis, treatment, and/or prognosis of a patient. Furthermore, because the fundamental goal of a billing dataset is to acquire the highest rate of reimbursement for the health care facility, the diagnoses listed for a patient may not accurately reflect the treatment or immediate threat to life. The combination of these factors relating to the use of billing datasets may result in misclassification and/or omission of heavy metal poisoning cases (Friedman et al., 2014; Krajewski and Friedman, 2015).

Additionally, the hospital discharge data does not capture data on the source of exposure or biological measurements in the body for heavy metals. This makes it difficult to discern patients being screened for heavy metal exposures from those being treated for adverse effects from these exposures. However, based on our data the majority had a primary diagnosis or reason for a visit related to heavy metal exposures. This indicates, that at least among the inpatient cases, these are likely cases being treated for adverse health effects related to heavy metal exposure. But the source is unknown. The exposure to heavy metals could be from occupational sources which seems most likely for adults, but because the zip code information is based on the patient's residence, it is hard to characterize spatial clustering in relation to known worksites or distinguish between combined environmental and occupational sources (Agency for Toxic Substances and Disease Registry, 2007b).

7.1.3 Implications

Heavy metals are ubiquitous in the environment, so exposure to low-levels in the general population is expected. For those that live near or work in industries that use or process heavy metals, the exposures would be higher than seen in the general population. However, because of the adverse health effects associated with exposure to heavy metals, there are occupational and environmental regulations and standards for heavy metals exposures. These regulations and standards are meant to minimize exposure and protect health, and as a result, there should be only limited numbers of health related events, driven by occupational exposures. From this analysis, there were 739 heavy metal poisoning cases treated in Illinois hospitals, and just under a third of

those cases (30.6%) were children. This implies that there are more than just small events of occupational exposures or acute occupational events (accidents) that result in treatment at a hospital.

Lead was the most commonly seen heavy metal exposure in children, this indicates that there are still issues surrounding exposure to lead. State childhood blood lead surveillance programs, in part, aim to identify high-risk geographic areas and populations, identify children at risk for targeted testing and resource allocation, and identify emerging sources of exposure (Centers for Disease Control and Prevention, 2016a). However, funding for this program is only available in 30 states, including Washington DC, and five major cities (Centers for Disease Control and Prevention, 2016a). In addition, based on CDC's compilation of state data for childhood blood lead levels, approximately 10% of children were tested for lead in the US (Centers for Disease Control and Prevention, 2016a). This is just one example of well-established public health surveillance program, particularly for a heavy metal (lead) exposure, and there are still gaps that need to be addressed.

Keeping the example of childhood blood lead level surveillance programs in mind and the use of NHANES and NPDS as current surveillance tools, there potential alternatives to monitor exposure to heavy metals. An ideal surveillance system for heavy metals would use a complimentary approach to combine the strengths of hospital discharge data, national health survey, and poisoning surveillance tools. This system would include demographics, source and location of exposure, biomonitoring data, health outcomes (diagnoses, hospital procedures, length of stay, discharge status), and economics (hospital charges, payer source). Having source and location of exposure

would identify geographic locations or occupational settings where regulations for heavy metals are being violated or poorly monitored and provide the necessary information to identify spatial clusters of these cases. Biomonitoring data provides comprehensive information on exposure, but also provides the necessary data to create or modify environmental and occupational health policies for exposures to heavy metals. Health outcomes data offers insights into treatment(s) of these cases and potential for reducing length of stay. Payor source provides information on if these cases are occupational (workers' compensation) or low-income (Medicaid or Medicare), which can help to target industries that are not compliant with occupational regulations, or in areas with limited financial resources to target prevention needs. A combination of all these factors would provide a comprehensive understanding of how populations are being exposed to heavy metals, provide targeted prevention strategies, and create or modify public health policies to further reduce exposure to heavy metals, with the potential of reducing treatment costs and economic burden for treating these cases.

7.2 Validity and Reliability of Biosensor Methodology for the Detection of Cadmium and Lead in Drinking Water

The field-based fluorimetric biosensor system, ANDalyze, used to quantify known concentrations of cadmium and lead in two different water types showed inconsistent performance. Overall, the method was positively biased for cadmium (Table XIV) and negative biased for lead in deionized water (Table XV); in tap water, the method was unable to detect lead at concentrations between 5 and 25 ppb (Table XV). The patterns quantitatively observed in the data were confirmed by statistical analysis. For cadmium, there were no samples measured by the ANDalyze method that were within $\pm 15\%$ of

the standard concentration for both deionized water and tap water. For lead in deionized water, 44% (N=8) samples were correctly identified by ANDalyze within $\pm 15\%$ of the standard concentration; however, in tap water, no samples measured by the ANDalyze method that were within $\pm 15\%$ of the standard concentration.

Variability is inherent in this biosensor method due to the fact that no two biosensors are exactly the same (Lu, 2009). ANDalyze reports the accuracy of the biosensor system as $\pm 15\%$ (Dugan, 2015). To minimize variability and confirm with manufacturer specifications, we measured water quality parameters (pH, temperature, turbidity, conductivity), filtered tap water, controlled incubation time, and used plastic sample collection bottles (Van Geen et al., 2005). Many of these factors are known to affect the quantification of heavy metals analyzed by a variety of methods, including ANDalyze (Lu, 2009). However, field kits are designed to work under “real-world” conditions, not laboratory controlled environments with deionized water media only. It is necessary, from a practical standpoint, to verify performance of these kits in target media, such as tap water from different water systems and private wells. This gives more confidence that field performance will match laboratory performance.

The presence of multiple metals in a water sample can interfere in the quantification of the target metal ion. ANDalyze tested and reported the levels for 15 chemicals that resulted in a $\pm 10\%$ change in the measured lead concentration. Six of these metals (cadmium, copper, iron, lead, mercury, and zinc) were found that all were present below the interference level. We were unable to quantify the other nine chemicals: aluminum, ammonium, calcium, carbonate, chloride, magnesium,

manganese, nitrate, phosphate, and sulfate; thus, these ions could potentially cause interference and inconsistency in the measured cadmium and lead concentrations.

Although the use of biosensors for the detection of heavy metals in water samples has been tested for several decades, published method performance studies in field settings are sparse (Bentley et al., 2001; Raja and Selvam, 2011; Rategarpanah et al., 2013; Guo et al., 2015; Li et al., 2015). A previous US EPA Environmental Technology Verification (ETV) report tested the ANDalyze system for the detection of lead in drinking water and concluded that method performance was subject to water quality characteristics dictated by environmental conditions and water quality characteristics artificially imparted on synthetic environmental or laboratory samples (Yates et al, 2013), similar to the results of this study. In addition, there was a major limitation with the ETV report: if on-site calibration failed after the second attempt or if acceptance criteria (quality control) was not met, samples were not analyzed, and the number of samples that did not meet this criteria was not reported (Yates et al, 2013). The number of samples unable to be analyzed is equally important to the number that were analyzed. The reported accuracy and precision in this report may not be accurate, as they are reflecting the total number of samples that met acceptance criteria rather than the total of samples attempted to be sampled.

7.2.1 Limitations

Future analyses would benefit from an increased number of standard concentrations for cadmium and lead, as well as other metals. The biosensor system has other heavy metal-specific sensors available, and the performance of those sensors

should also be evaluated, as the CVs and percent errors of these other metal-specific sensors could be different from what was found in the cadmium and lead sensors in this research.

This study used a single source of purified deionized water and drinking water, and additional samples from different locations and other water sources, such as well water, would have enhanced the findings of this research. The quality of drinking water, including the presence of contaminants that interfere with sensor performance, may differ by infrastructure and water purification process on the detection of the sensor (Guidotti et al., 2007; Renner, 2009). However, similar results for other City of Chicago tap water sources would be expected, based on the annual drinking water quality report released by the city. The City of Chicago water quality for lead in drinking water is below the US EPA TT action level of 15 ppb lead (City of Chicago, 2015). Furthermore, although there was a single source of purified deionized water, this is not typical source of drinking water for the general population. The ANDalyze system was designed to be able to detect metals in drinking water sources and not just controlled laboratory samples, and as such, evaluations for field-based detection methods for drinking water should be designed for real-world conditions with multiple contaminants at trace (low-doses) concentrations.

Three different incubation times were considered, in order to determine if incubation of samples would impact the detection of cadmium or lead by the biosensor system. Baghel et al. found that the greater the concentration, the more rapid the detection time (Baghel et al., 2007). Van Geen et al. found that when using the Hach EZ arsenic test kit, increasing the reaction time greatly increased the measurement

accuracy (Van Geen et al., 2005). Additionally, exploration of storage and additional incubation procedures are needed to determine those that will result in the highest degree of accuracy for different types of water samples. However, in this study, the results did not vary among incubation times by water type.

One specific limitation of the ANDalyze system is that the lower limit of detection of 0.1 ppm for the cadmium sensor is greater than the US EPA MCL for cadmium of 0.005 ppm (United States Environmental Protection Agency, 2014a). In terms of efficacy for field-based detection methods, the equipment should be able to detect concentrations of contaminants in real-world settings and at levels set by federal, state, and/or local drinking water regulations. The fact that the lower limit of detection for cadmium is 20 times the MCL, this sensor is not relevant for use to assess the quality of drinking water with respect to cadmium.

The ANDalyze system used for this analysis has since been replaced with a newer model. The manufacturer states that this new model “has twice the sensitivity” (ANDalyze, 2014). Because of this change, the results of this study could potentially be different and the detection of the cadmium and lead in both deionized water and tap water could be improved with the new model.

Despite these limitations, there are several strengths to this study. The standard concentrations for cadmium and lead were verified by an external laboratory with a gold-standard detection method. The analysis for the detection of lead drinking water was similar to a previous US EPA ETV report and the results from that report and from this research found that the ANDalyze system was subject to water quality

characteristics dictated by environmental conditions and water quality characteristics of environmental or laboratory samples.

7.3 Reliability of Commercially Available Field Kits for the Detection of Lead in Drinking Water with Lead and Lead-Mixtures

The three field-based kits evaluated for the detection of lead in drinking water varied in performance, among the kits, when lead was present alone or in mixtures, and when lead was in deionized or tap water. In tap water, the First Alert and WaterSafe kits resulted in errors as the lead only standard concentrations were 13 ppb and 15 ppb, where 15 ppb is the method detection limit and should provide a positive result. Overall, for all three test kits, the performance for lead mixtures in tap water were highly variable, with the First Alert and WaterSafe kits resulting in mostly all FNs and 64% of the Pro-Lab measured lead concentrations in tap water being outside a $\pm 15\%$ accuracy range.

In general, the way in which water is collected can impact the results for the detection of lead, which ultimately is an issue for exposure assessment (Renner, 2010). In order to minimize the impact on water collection methods on the method performance, the manufacturer's instructions were followed, when available. The differences in how water is collected and sampled have been at the center of debate for a number of years (Renner, 2010). One of the sampling parameters that has been questioned is the water temperature (Renner, 2010). Cold water is typically sampled, as was specified by the Pro-Lab test kit (Pro-Lab, 2013; Renner, 2010). However, the use of cold water samples may not be accurate collection technique as more lead may be released into water from hot water (Renner, 2010). Another issue is the collection

tubes/containers. Like listed in the Pro-Lab instructions, the water was first collected in one container and then poured into another (Pro-Lab, 2013; Renner, 2010). However, during this process, heavy lead particles may sink to the bottom of the container and may not be present in the sample (Renner, 2010). In order to correct this potential issue, the water collected for the Pro-Lab kits was stirred (as directed by the package insert), while the samples for the First Alert and WaterSafe kits was gently stirred before placing the sample in the vials for testing. Furthermore, the stream/speed of the water from the tap may also cause differences in measured lead concentrations, as more lead may be released with a heavier flow of water (Renner, 2010). The Pro-Lab kit specifically stated for the cold water to be turned on to a slow trickle, and for consistency, the water used in the First Alert and WaterSafe kits also was collected from a slow trickle, after a slow trickle used to flush the cold tap (Pro-Lab, 2013).

The Pro-Lab test kits are a mail-away kit, and we did not receive results for all samples submitted. In total, 27 samples were mailed to Pro-Lab for analysis. However, confirmation and results for 23 samples were received from Pro-Lab, two samples never received, and two samples received for radon testing. Attempts to contact Pro-Lab about how two samples were labeled for radon testing and two samples were not received altogether went unanswered.

In addition, two samples were above the detection range of 15 ppb, at 18 ppb and 33 ppb, respectively (Appendix C), but the determination was that the samples passed: "All of the tested parameters meet the Environmental Protection Agency's (EPA) Primary (health-related) Drinking Water Standards maximum contaminant level goals for human consumption, as well as the FHA/HUD general established statutes for

maximum contaminant levels for water potability” (Pro-Lab, 2016 in Appendix C).

Following inquiries about how the determination of “Pass” was made, the company reissued the certificate of analysis and changed the determination to “Fail” (Appendix C). Although the results were reissued with the corrected determination, this can result in confusion and a false sense of security for the general consumer about the state of their drinking water. Furthermore, each certificate of water analysis is signed by a quality control manager. When the inquiry was made about how the determinations are made, a reissued report was sent within seconds by a customer service representative. The fact that the report could be reissued so quickly by the customer service representative, with a signature from the quality control manager, leads to questions about what are the standards for the quality control measures taken at the company, how often does do certificate of water analysis reports have to be reissued, and what happens to consumers who do not follow up on the disparities in the results and the acceptable range with a “pass” determination. Multiple efforts were made to contact the quality control manager through customer service, but no information on how to contact the quality control manager was provided.

The results for the statistical measures of performance evaluating the three field-based test kits can be misleading, making it appear that the kits perform better than they actually do. For example, there were no FPs for the First Alert and WaterSafe kits, so both the specificity and precision were 100%, and a false positive rate could not be calculated. The absence of FPs in the measured lead concentrations could mean that the kits can accurately detect samples without or lower detectable concentrations of lead; however, based on the results from this research, more samples would be need to

be tested to draw further conclusions. This needs to be considered when evaluating the performance and results. The number of samples needed could be calculated using sensitivity and specificity to generate a receiving operating characteristic (ROC) curve, and then calculating the area under the curve (AUC).

In the presence of copper and/or iron, the concentration of lead was masked; in the Pro-Lab results, the lead concentration was lower than expected and in the WaterSafe and First Alert kits, lead was not detected. Both the ICP-MS analysis and the Pro-Lab results measured similar concentrations of lead when it was in mixture with copper and/or iron, meaning that the two methods have similar accuracy in detecting lead in a mixture. For the standard with lead only at 15 ppb, the Pro-Lab analysis and the ICP-MS measured the concentration as 20 ppb and 19 ppb, respectively; however, when in mixture with copper and/or iron, the Pro-Lab and ICP-MS results ranged from as 9 ppb to 14 ppb. It is unlikely that this difference reflects an error in the standard solution, as all standards with 15 ppb lead were from the same serial dilution sample. The result of copper and/or iron acting as. Whether this antagonism effect between lead and copper and/or iron is functional (counterbalancing of the toxic effects), chemical (copper, iron, and/or another contaminant react with lead to reduce toxicity), receptor (lead and another contaminant are bound to a receptor, and the concentration of lead is lessened) or dispositional (the disposition of lead is altered, i.e. no longer free lead, so that the concentration is diminished) is unknown, but it warrants further analysis, as this means measurements of lead in drinking water are likely underestimated.

There are limited studies on the effects of heavy metal mixtures in drinking water and the effect on detection; however, there are numerous studies that show explain

observed correlations between lead and iron in drinking water through adsorption mechanisms using synthesized iron oxides (Trueman and Gagnon, 2016). As a way to remove heavy metals from drinking water, nanosized metal oxides have been used experimentally (Hua et al., 2012). In particular, iron-based metal oxides were shown to bind with target metals, like lead and copper, for sorption and removal (Hua et al., 2012). In addition, high iron often accompanies elevated lead in drinking water (Trueman and Gagnon, 2016). The propensity for lead to adsorb onto iron surfaces is due to high solid-water partition coefficient (Trueman and Gagnon, 2016). Removal of heavy metals from drinking water is ongoing exploration into technologies and methodologies that could aid in this process. Studies have shown that the ability for lead to be bound to iron is an efficient strategy for removal (Barakat, 2011; Fu and Wang, 2011; Trueman and Gagnon, 2016), however, these studies also show that this relationship could have the potential to interfere with detection of lead in drinking water.

The three kits evaluated here were specifically chosen for their availability and affordability. The test kits ranged in price from (approximately) \$10 to \$50, with estimated annual sales for the companies as follows: \$662 million for First Alert, \$2.27 million for WaterSafe's parent company, and \$78 million for Pro-Lab (Hoover's Inc., 2016a; 2016b, 2016c). These at-home test kits provide empowerment to the consumer, especially in situations like that of Flint, MI. The availability of these products offer consumers that ability to test their own drinking water and get their own results, amidst conflicting reports on the quality their drinking water, causing distrust of government agencies in charge of water testing and monitoring. However, the results here indicate that there is high variability among test performance and the interpretation of results,

which can only cause further frustration and confusion. When buying these products, consumers have the expectation that these multimillion dollar companies have properly tested their products to ensure performance accuracy and reliable results, but the results of this study question the accuracy of detection limits of test strips and within lead mixtures.

7.3.1 Limitations

The process in which water is collection can cause differences in measured concentrations. The use of hot or cold water may draw water through the tap that have different levels of lead. As the Pro-Lab kit specifically specified cold water to be used, the same process and temperature was used for the First Alert and WaterSafe kits for consistency among the samples. Another collection parameter that could be tested is the difference in the stream for collection, meaning if a heavier stream of water results in different measured concentrations than a slow trickle. Therefore, having more samples which tested these differences would provide a more comprehensive understanding of how collection techniques effect measured concentrations of lead in a sample and which technique could potentially provide a more representative sample estimate of lead from drinking water sources.

In addition to collection techniques, this research would have been enhanced by the use of drinking water from different locations in Chicago and from other cities and states, as well as from other water sources, such as well. Older and/or poorly maintained infrastructure could potentially cause increased levels of contaminants (lead and other heavy metals) in the drinking water supply, which in turn could interfere with

the field kit's precision to accurately detect heavy metals (Guidotti et al., 2007; Renner, 2009). In addition, water purification processes at water treatment plants and in the home can impact the contaminants (biological and chemical) in the drinking water supply, which could alter the measured concentrations of lead by the field kits (Guidotti et al., 2007; Renner, 2009).

Even with the limitations of the study, the results from this research provides insight on the performance of commercial available field kits. The results of these kits were compared with that of a gold-standard laboratory method, ICP-MS, and one of the field kits provided results from ICP-AES laboratory analysis. The standard concentrations evaluated in this study took into account the performance of these kits at US EPA's MCLs and TT action levels. Furthermore, the evaluation of these test kits for lead in mixtures has not previously been done, and these results provide the need for further evaluation how contaminants in mixtures may alter the detection of a single analyte.

7.3.2 Implications

The results of this study raise questions about the accuracy and reliability of these three field kits. For First Alert and WaterSafe, as the standard concentration approached the detection limit of 15 ppb lead, the test strips were prone to errors, meaning that the result of the test strip was an error. Based on the results of this study, for consumers who are purchasing these test kits because they are afraid that their drinking water may be near, at, or over 15 ppb, the TT action level for lead, the results can yield error readings. Additionally, if copper and/or iron are present in the drinking,

the result for lead may result in a false negative, give consumers the belief that their drinking water is safe to drink. The resulting public health impact is significant, as people could be drinking water that is above the TT action level and could result in adverse health outcomes (neurodevelopmental effects), especially for children.

Pro-Lab was unreliable for receiving, processing, and providing accurate results. The Pro-Lab kit was the most expensive of the three kits tested. The fact that the results are from a laboratory analysis may provide consumers with confidence that the results are accurate because the test is done in a laboratory, presumably with trained technicians. However, this not this experience in our case. Not only were some of the samples not received by Pro-Lab (even though the laboratory analysis was paid for), there were two samples that were not even processed for the correct analysis. In addition, two samples yielded initially results of “Pass” when the measured concentration was above the TT action level. Furthermore, as seen with the ICP-MS results and the high number of FNs from the First Alert and WaterSafe test kits, the ICP-AES analysis provided by Pro-Lab found that in the presence of lead mixtures, the measured concentrations are below the standard. Thus, when results were provided from Pro-Lab, there is questions regarding their quality control how the determination results are monitored and reported.

Given these issues and based on the results of this study, it is not recommended that consumers use these field kits to ascertain their drinking water quality for lead. All three field kits had issues detecting lead in mixtures, and if the presence of multiple heavy metals can cause interference, then the detection methods should be reevaluated by the manufacturers and external laboratories for their accuracy in

mixtures. This information should be provided to consumers, alerting them to the possibility of the performance changes for the detection of lead with heavy metal mixtures. In addition, the manufacturers should also provide more details on how to interpret the results, and be able to put into context what the results mean. There should also be more resources available from public health officials and health departments about recommended, certified laboratories – either at a local or state agency or external laboratories. Inaccurate interpretation of results or poor performance of these test kits could result in major public health ramifications, resulting in people being exposed to lead in their drinking water, when they believe their water is safe to drink.

7.4 Summary of Conclusions

Hospital discharge data has not previously been utilized for surveillance of heavy metals exposures resulting in hospitalizations in the general population. However, there are some limitations with hospital discharge data as a surveillance tool. Although the hospital data captures exposure, health outcomes (diagnoses, hospital procedures, length of stay, discharge status), and economics (hospital charges, payer source), which are not recorded in national health survey data, it does not provide biological measurements nor the source of exposure for the heavy metals. Hospital discharge data likely captures predominately the most severe or acute cases of exposure to heavy metals rather than chronic or low-dose exposures that do not result in signs or symptoms, possibly from environmental sources. However, using billing data in conjunction with other surveillance tools will help build a broader picture of potential

heavy metal exposures in a population and possibly identify “outbreaks” within the population. The economic burden of heavy metal poisonings resulting in treatment at a hospital is substantial in Illinois alone and having data sources of exposures available would help in identifying areas or occupational settings where regulations are being violated or poorly monitored and provide the necessary information to identify spatial clusters of these cases. This information could in turn help environmental public health officials and industries to monitor and ensure compliance to reduce the burden of heavy metal poisonings resulting in hospitalizations.

The biosensor system evaluated in this research, ANDalyze, has the potential to offer time-efficient and relatively inexpensive analysis of heavy metals, such as cadmium and lead, in drinking water. However, the ANDalyze fluorimeter (AND1000) did not prove to be a reliable device for measuring cadmium and lead in our hands. The ANDalyze system completely failed to detect lead in tap water samples at standard concentrations of 5-25 ppb. The analysis for the detection of lead in drinking water was similar to a previous US EPA ETV report and the results from that report and from this research found that accuracy of the ANDalyze system was affected by water quality characteristics dictated by environmental conditions. Further study is specifically warranted to explore whether the poor quantification of lead in tap water in this study is unique to the tap used or to drinking water in the City of Chicago. This study confirms that testing of analytical methods intended for analysis of environmental samples should go beyond laboratory conditions, and consider performance in environmental matrices and field deployment. Future research is needed to study reliability and validity of this biosensor system for heavy metal mixtures.

With the increasing attention on lead in drinking water supplies, having a reliable field-based method for detecting all heavy metals in drinking water that can be used by community members to test their water supply has public health value. The current literature evaluating field-based tests for detecting heavy metals in drinking water is largely focused on the detection of arsenic, not lead. The three field-based kits evaluated in this research have the potential to offer time-efficient, low-cost access to information about lead levels in drinking water for the public. However, although the three field-based kits had higher performance accuracy for detecting lead when it is the sole contaminant in tap water, they fail to reliably and accurately detect lead when it is present in a mixture with copper and/or iron. Further research is needed to better understand why these methods were adversely impacted by the presence of mixtures. In the wake of Flint, MI, the awareness about lead in drinking water has been heightened. Consumers who are concerned about their drinking water can go buy one of these relatively inexpensive “at-home” kits to provide a sense of security. However, all three test kits had their issues; the interpretation of the test strips from First Alert and WaterSafe are dependent on subjectivity and there were numerous errors as the standard concentration approached the detection limit, while Pro-Lab had poor reliability with receiving and processing samples, and with quality control of the results. As indicated by the results of this study, the presence of other heavy metals may cause interference with accurately detecting lead in drinking water, providing consumers with a false sense of security, with larger public health implications overall.

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APPENDICES

APPENDIX A

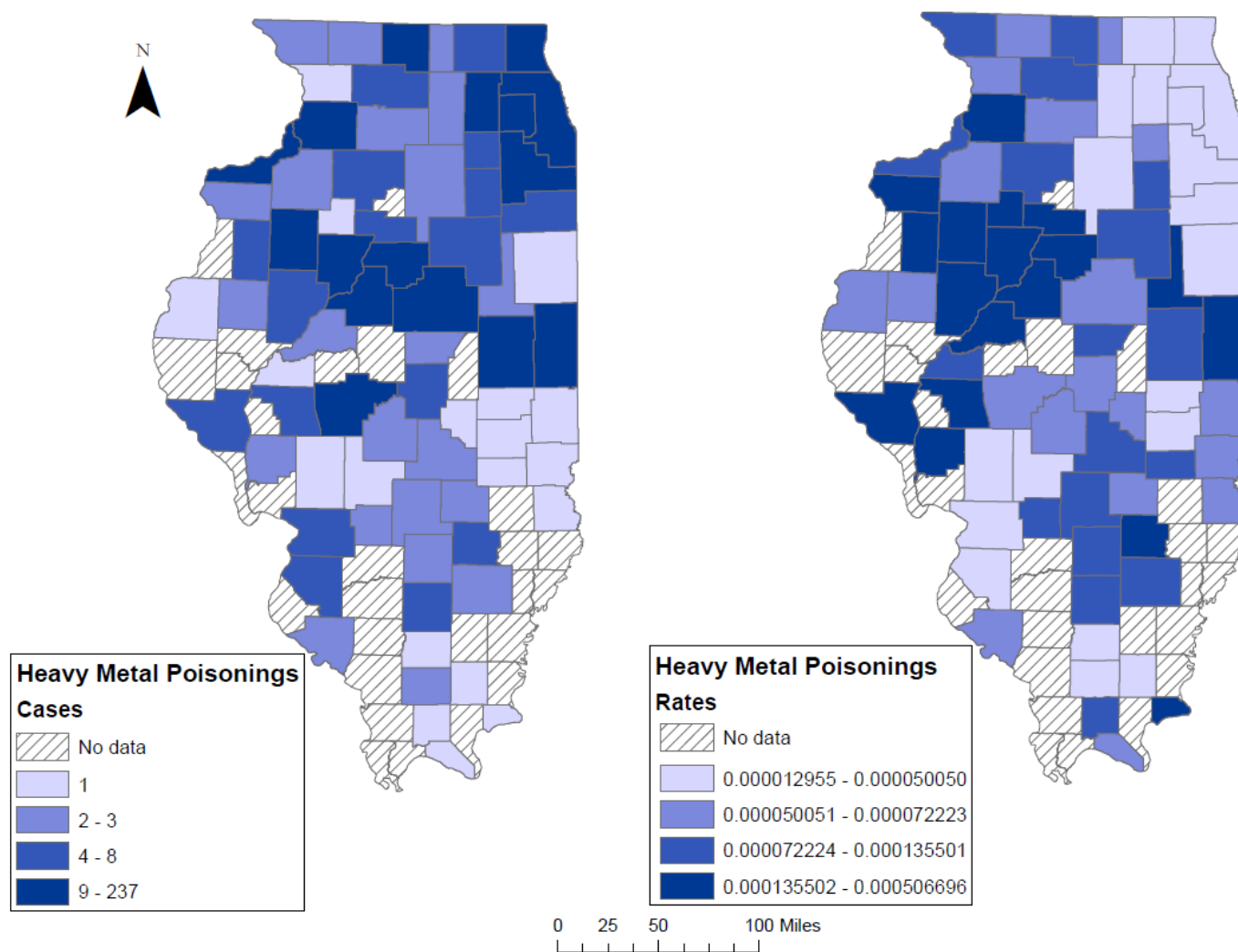


Figure 4: Overall Heavy Metal Poisoning Cases Treated in Illinois Hospitals, 2010-2013 by Frequency and Rates.^a

^a Rates were standardized with the 2010 Illinois Census population numbers.

Appendix A (continued)

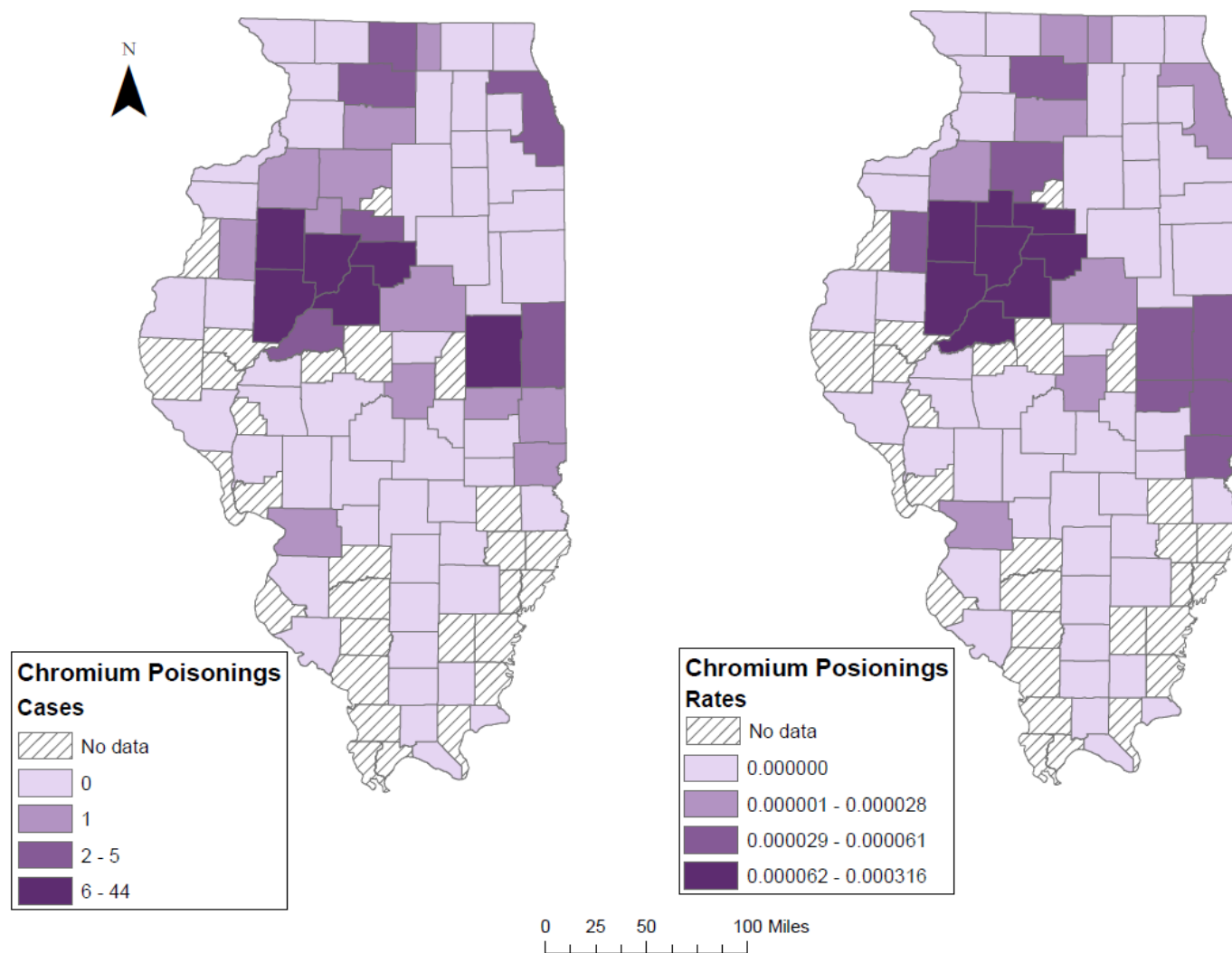


Figure 5: Chromium Poisoning Cases Treated in Illinois Hospitals, 2010-2013 by Frequency and Rates.^a

^a Rates were standardized with the 2010 Illinois Census population numbers.

Appendix A (continued)

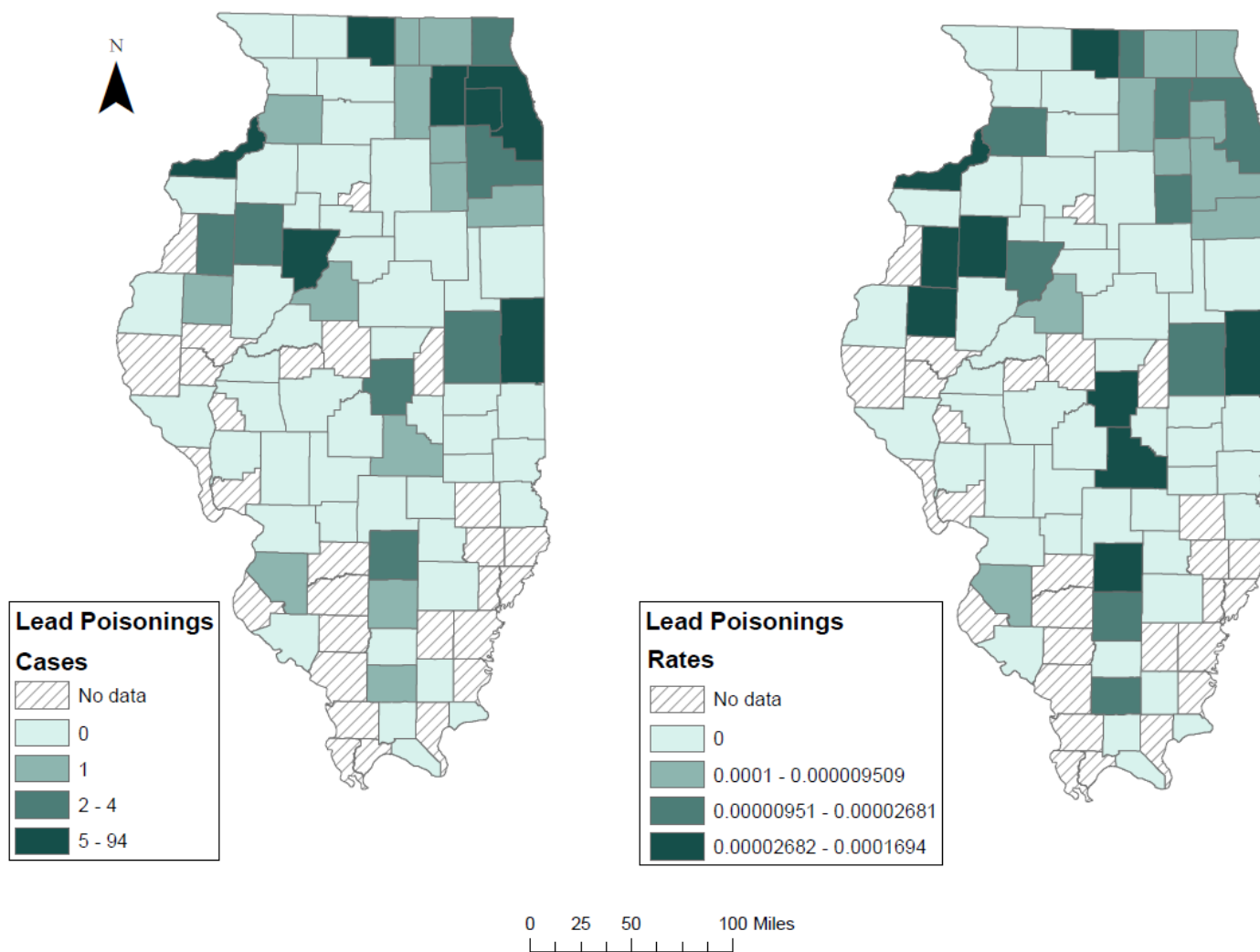


Figure 6: Lead Poisoning Cases Treated in Illinois Hospitals, 2010-2013 by Frequency and Rates.^a

^a Rates were standardized with the 2010 Illinois Census population numbers.

Appendix A (continued)

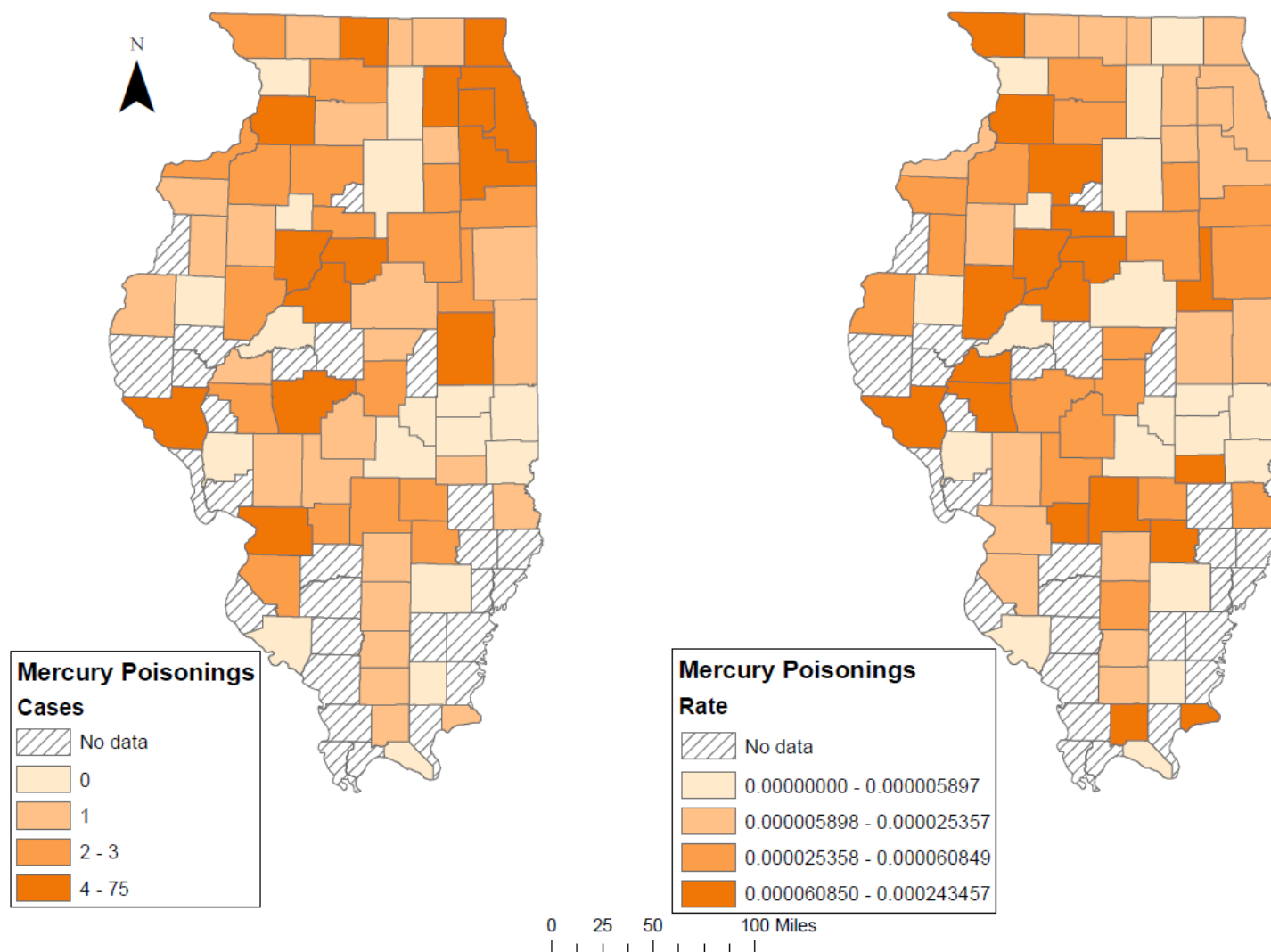


Figure 7: Mercury Poisoning Cases Treated in Illinois Hospitals, 2010-2013 by Frequency and Rates.^a

^a Rates were standardized with the 2010 Illinois Census population numbers.

APPENDIX B

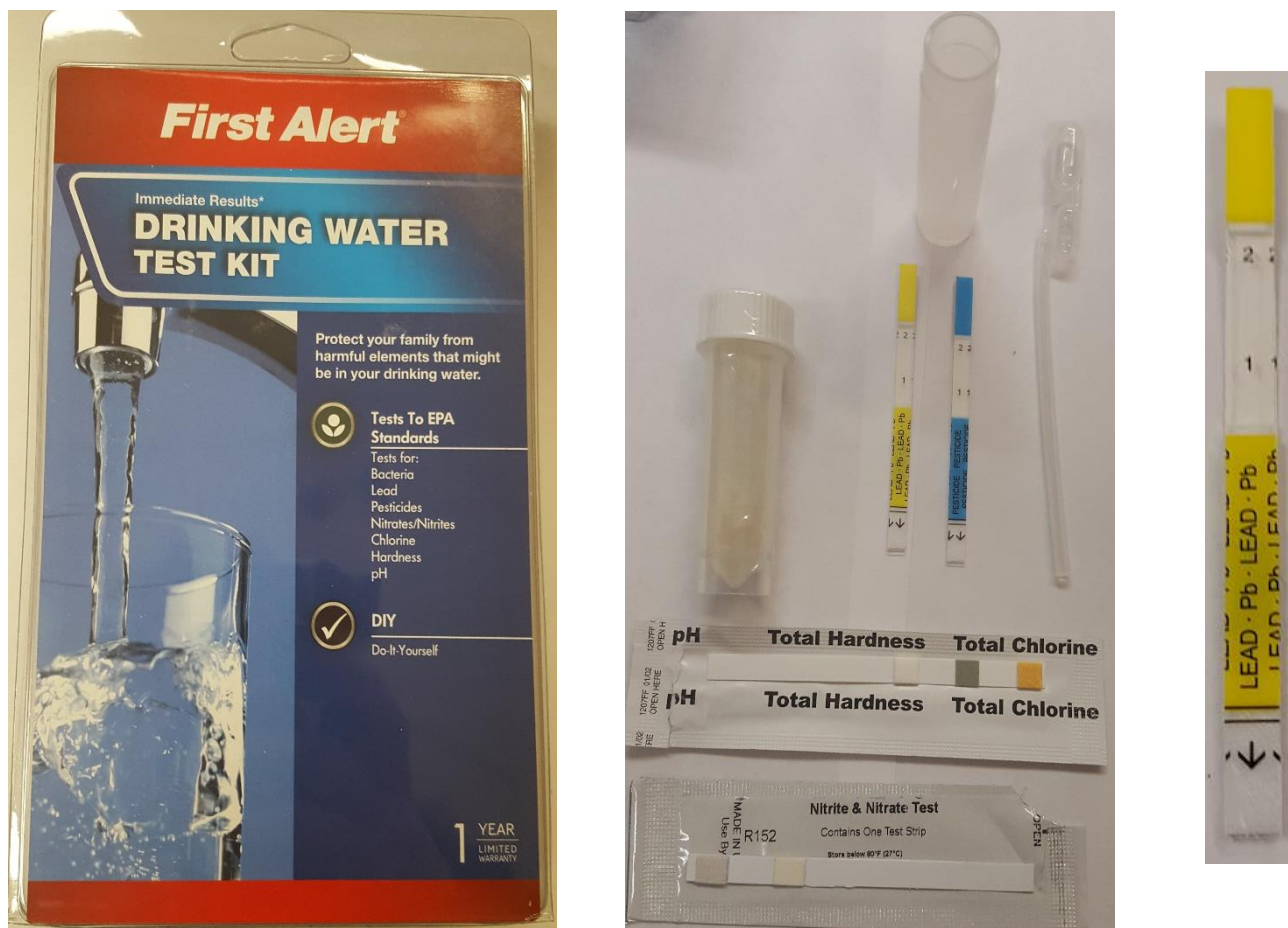


Figure 8: First Alert Drinking Water Test Kit.

APPENDIX B (continued)

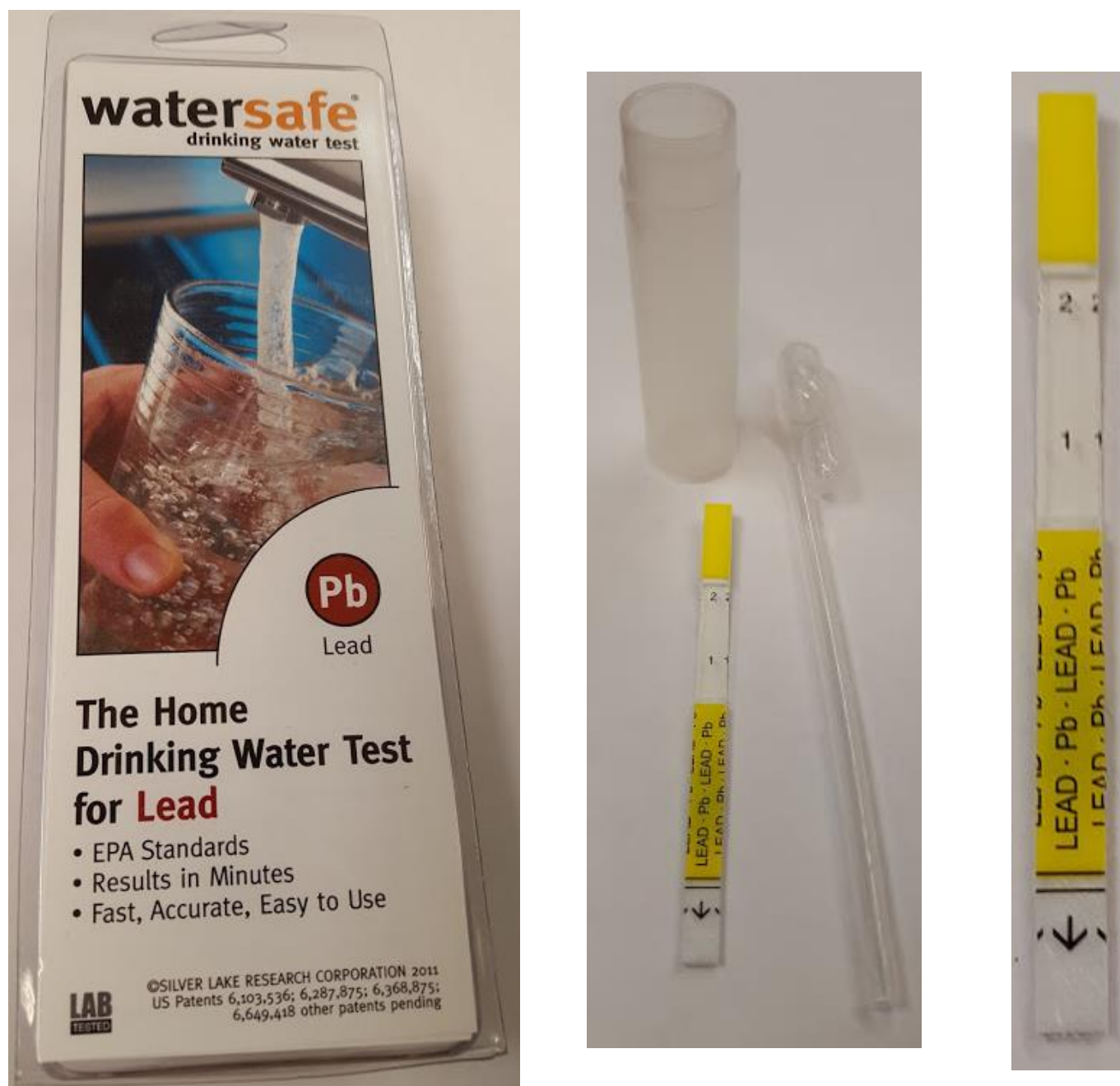


Figure 9: WaterSafe Drinking Water Test for Lead.

APPENDIX B (continued)

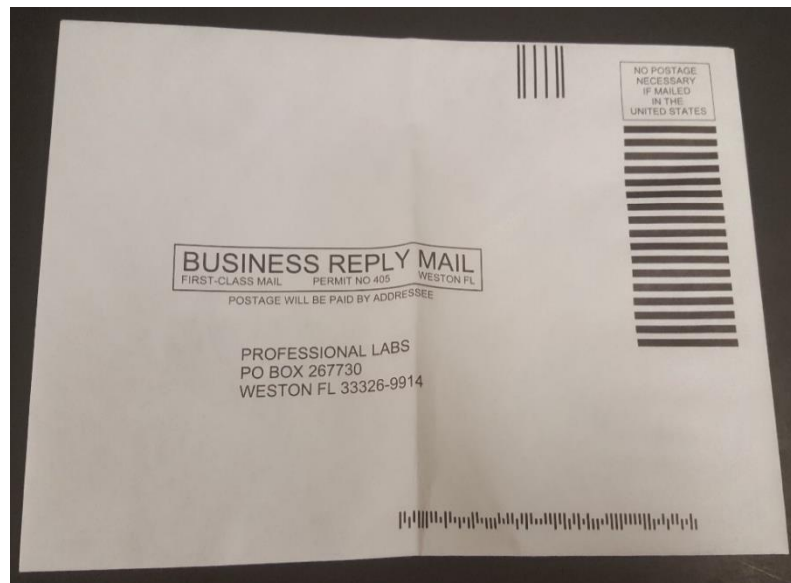
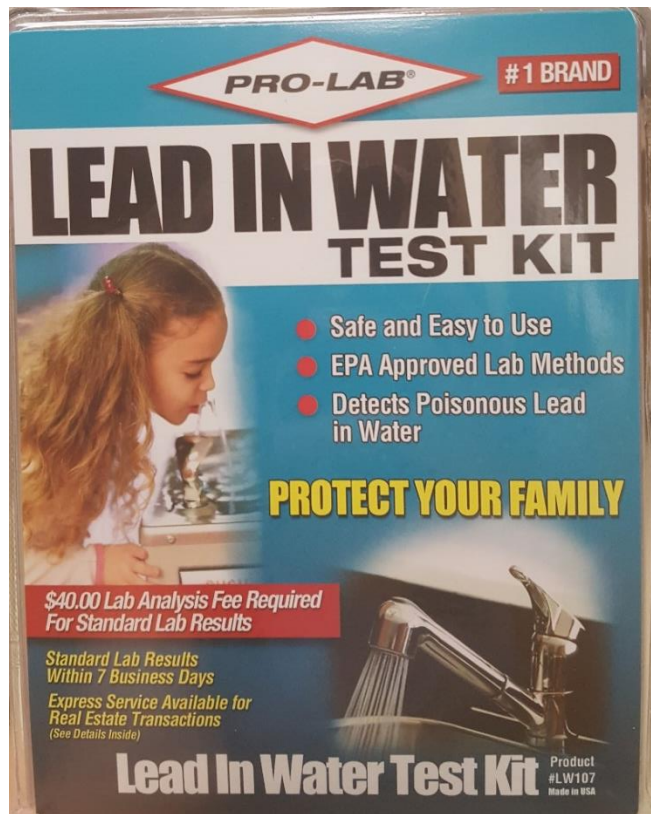



Figure 10: Pro-Lab Lead in Water Test Kit.

APPENDIX C



1675 North Commerce Parkway, Weston, FL 33326 (954) 384-4446

Test Address:

[Redacted]

Test Address:

[Redacted]

Email: [Redacted]

Phone: [Redacted]

Test / COC #: 931489

Sample ID: 031416-0138

Sample Date: March 7, 2016

Date Received: March 14, 2016

Analysis Date: March 23, 2016

Report Date: March 23, 2016

Sample Location: [Redacted] 35

CERTIFICATE OF WATER ANALYSIS


TEST ITEM	RESULT	ACCEPTABLE RANGE	DETERMINATION
LEAD	0.009 ppm	0 to 0.015 ppm	Pass

BDL = Below detection limit; cfu = colony forming units; mg/L = milligrams per liter; ntu = nephelometric turbidity units
 pH = acidity/alkalinity units; ppm = parts per million; ppb = parts per billion; µS/cm = microSiemens per centimeter
 U = Indicates that the compound was analyzed for but not detected. The value associated with the qualifier shall be the laboratory method detection limit.
 DET = Analyte detected; ND = Analyte not detected at or above the detection limit; NR = Not reported; dry = Sample results reported on a dry weight basis.
 RPT = Relative percent difference; V = Indicates that the analyte was detected in both the sample and the associated method blank.
 I = The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.
 Z = too many colonies were present for accurate counting

CONCLUSION: PASS

All of the tested parameters meet the Environmental Protection Agency's (EPA) Primary (health-related) Drinking Water Standards maximum contaminant level goals for human consumption, as well as the FHA/HUD general established statutes for maximum contaminant levels for water potability.

The EPA is responsible for the National Primary Drinking Water Regulations, which are health-related standards that establish the Maximum Contaminant Levels. MCLs are the maximum permissible level of a contaminant in water delivered to users of a public water system. MCLs are enforceable under the Safe Drinking Water Act. National Primary Drinking Water Regulations can be found on the web at: <http://water.epa.gov/drink/contaminants/index.cfm>




Carlos Ochoa, Quality Control Manager

The above results are provided by Florida Environmental, NELAC #FL-86006, EPA Lab Code FL00095

Figure 11: Pro-Lab Certificate of Water Analysis for Known Lead Concentration of 5 ppb in Tap Water.

APPENDIX C (continued)



1675 North Commerce Parkway, Weston, FL 33326 (954) 384-4446

Test Address:

[Redacted]

Email: [Redacted]
Phone: [Redacted]

Test / COC #: 931504
Sample ID: 031416-0169

Sample Date: March 7, 2016
Date Received: March 14, 2016
Analysis Date:
Report Date: March 23, 2016
Sample Location: [Redacted] 34

CERTIFICATE OF WATER ANALYSIS


TEST ITEM	RESULT	ACCEPTABLE RANGE	DETERMINATION
LEAD	0.018 ppm	0 to 0.015 ppm	Pass

BDL = Below detection limit; cfu = colony forming units; mg/L = milligrams per liter; ntu = nephelometric turbidity units
 pH = acidity/alkalinity units; ppm = parts per million; ppb = parts per billion; µS/cm = microSiemens per centimeter
 U = Indicates that the compound was analyzed for but not detected. The value associated with the qualifier shall be the laboratory method detection limit.
 DET = Analyte detected; ND = Analyte not detected at or above the detection limit; NR = Not reported; dry = Sample results reported on a dry weight basis.
 RPT = Relative percent difference; V = Indicates that the analyte was detected in both the sample and the associated method blank.
 I = The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.
 Z = too many colonies were present for accurate counting

CONCLUSION: PASS

All of the tested parameters meet the Environmental Protection Agency's (EPA) Primary (health-related) Drinking Water Standards maximum contaminant level goals for human consumption, as well as the FHA/HUD general established statutes for maximum contaminant levels for water potability.


The EPA is responsible for the National Primary Drinking Water Regulations, which are health-related standards that establish the Maximum Contaminant Levels. MCLs are the maximum permissible level of a contaminant in water delivered to users of a public water system. MCLs are enforceable under the Safe Drinking Water Act. National Primary Drinking Water Regulations can be found on the web at: <http://water.epa.gov/drink/contaminants/index.cfm>


 Carlos Ochoa, Quality Control Manager

The above results are provided by Florida Environmental, NELAC #FL-88008, EPA Lab Code FL00095

Figure 12: Pro-Lab Certificate of Water Analysis for Expected Concentration of 15 ppb Lead and Determination of Pass.

APPENDIX C (continued)



1675 North Commerce Parkway, Weston, FL 33326 (954) 384-4446

Test Address:

Email: [REDACTED]

Phone: [REDACTED]

Test / COC #: 931470

Sample ID: 031416-0098

Sample Date: March 7, 2016

Date Received: March 14, 2016

Analysis Date:

Report Date: March 23, 2016

Sample Location: [REDACTED] 25

CERTIFICATE OF WATER ANALYSIS


TEST ITEM	RESULT	ACCEPTABLE RANGE	DETERMINATION
LEAD	0.033 ppm	0 to 0.015 ppm	Pass

BDL = Below detection limit; cfu = colony forming units; mg/L = milligrams per liter; ntu = nephelometric turbidity units
 pH = acidity/alkalinity units; ppm = parts per million; ppb = parts per billion; µS/cm = microSiemens per centimeter
 U = Indicates that the compound was analyzed for but not detected. The value associated with the qualifier shall be the laboratory method detection limit.
 DET = Analyte detected; ND = Analyte not detected at or above the detection limit; NR = Not reported; dry = Sample results reported on a dry weight basis.
 RPT = Relative percent difference; V = Indicates that the analyte was detected in both the sample and the associated method blank.
 I = The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.
 Z = too many colonies were present for accurate counting

CONCLUSION: PASS

All of the tested parameters meet the Environmental Protection Agency's (EPA) Primary (health-related) Drinking Water Standards maximum contaminant level goals for human consumption, as well as the FHA/HUD general established statutes for maximum contaminant levels for water potability.

The EPA is responsible for the National Primary Drinking Water Regulations, which are health-related standards that establish the Maximum Contaminant Levels. MCLs are the maximum permissible level of a contaminant in water delivered to users of a public water system. MCLs are enforceable under the Safe Drinking Water Act. National Primary Drinking Water Regulations can be found on the web at: <http://water.epa.gov/drink/contaminants/index.cfm>




Carlos Ochoa, Quality Control Manager

The above results are provided by Florida Environmental, NELAC #FL-86006, EPA Lab Code FL00095

Figure 13: Pro-Lab Certificate of Water Analysis for an Expected Concentration of 25 ppb Lead and Determination of Pass.

APPENDIX C (continued)



1675 North Commerce Parkway, Weston, FL 33326 (954) 384-4446

Test Address:

Test Address:

Email: [REDACTED]

Phone: [REDACTED]

Test / COC #: 931470

Sample ID: 031416-0098

Sample Date: March 7, 2016

Date Received: March 14, 2016

Analysis Date: March 23, 2016

Report Date: March 23, 2016

Sample Location: [REDACTED] 25

CERTIFICATE OF WATER ANALYSIS


TEST ITEM	RESULT	ACCEPTABLE RANGE	DETERMINATION
LEAD	0.033 ppm	0 to 0.015 ppm	*** FAIL ***

BDL = Below detection limit; cfu = colony forming units; mg/L = milligrams per liter; ntu = nephelometric turbidity units
 pH = acidity/alkalinity units; ppm = parts per million; ppb = parts per billion; µS/cm = microSiemens per centimeter
 U = Indicates that the compound was analyzed for but not detected. The value associated with the qualifier shall be the laboratory method detection limit.
 DET = Analyte detected; ND = Analyte not detected at or above the detection limit; NR = Not reported; dry = Sample results reported on a dry weight basis.
 RPT = Relative percent difference; V = Indicates that the analyte was detected in both the sample and the associated method blank.
 I = The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.
 Z = too many colonies were present for accurate counting

CONCLUSION: * FAIL *****

Some or all of the tested parameters do not meet the Environmental Protection Agency's (EPA) Primary (health-related) Drinking Water Standards maximum contaminant level goals for human consumption, as well as the FHA/HUD general established statutes for maximum contaminant levels (MCLs) for water potability.

The EPA is responsible for the National Primary Drinking Water Regulations, which are health-related standards that establish the Maximum Contaminant Levels. MCLs are the maximum permissible level of a contaminant in water delivered to users of a public water system. MCLs are enforceable under the Safe Drinking Water Act. National Primary Drinking Water Regulations can be found on the web at: <http://water.epa.gov/drink/contaminants/index.cfm>



Carlos Ochoa, Quality Control Manager

The above results are provided by Florida Environmental, NELAC #FL-98006, EPA Lab Code FL00095

Figure 14: Pro-Lab Reissued Certificate of Water Analysis for an Expected Concentration of 25 ppb Lead and Determination of Fail.

VITA

Alison K. Krajewski

Education

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|-----------|--|
| 2012-2016 | University of Illinois at Chicago, School of Public Health, Chicago, IL
PhD in Environmental and Occupational Health Sciences
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<i>Collateral:</i> Epidemiological and Research Methods |
| 2009-2011 | Boston University, School of Public Health, Boston, MA
MPH in Environmental Health
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| 2004-2009 | Michigan State University, Lyman Briggs College, East Lansing MI
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Research Experience

-
- | | |
|--------------|--|
| 2012-2015 | <i>Research Assistant</i> for Dr. Lee Friedman
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Teaching Experience

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- | | |
|------------|--|
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EOHS 400: Principles of Environmental Health
EOHS 418: Analysis of Water and Wastewater Analysis |
| 2015 | <i>Guest Speaker</i> , University of Illinois at Chicago, School of Public Health
IPHS 494: Determinants of Health, Cohort A and B
<i>"The Integration of Environmental and Community Health: A Case Study Example from Exposure Assessment to Community Outreach"</i> |
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PUB 370: Using the Public Health Toolbox |

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 EH 708: Introduction to Environmental Health

Professional Experience

2011-2012 *ATSDR (Agency for Toxic Substances and Disease Registry) Toxicology Health Educator*, Michigan Public Health Institute Affiliate Employee at Michigan Department of Health and Human Services, formerly Michigan Department of Community Health, Lansing, MI

Peer-Reviewed Publications

Krajewski AK and Friedman LS. Hospital outcomes and economic costs from poisoning cases in Illinois. *Clin Toxicol (Phila)*. 2015 Jun; 53(5): 433-445. Epub 2015 Apr 14.

Friedman LS, **Krajewski A**, Vannoy E, Allegretti A, and Wahl M. The association between US Poison Center assistance and length of stay and hospital charges. *Clin Toxicol (Phila)*. 2014 Mar; 52(3); 198-206.

Conference Presentations

- 2015 **Krajewski AK**, Jagai JS, and Heimler I. *Validation of a Biosensor Methodology for Detecting Heavy Metal Contamination in Drinking Water Sources*. Poster presented at American Public Health Association (APHA), 143rd Annual Meeting and Expo, Chicago, IL
- 2014 **Krajewski A**, Madigan D, Forst L, and Friedman L. *Leveraging Multiple Data Sources to Assist OSHA in Enforcement: Examining Illinois Workers' Compensation Data on Amputations*. Poster presented at International Commission on Occupational Health (ICOH), 24th International Epidemiology in Occupational Health (EPICOH) Conference Chicago, IL
- 2012 **Krajewski A**, Bush C, Groetsch K, and Gray J. *10 Mile Drain Superfund Site in St. Clair Shores, Macomb County, MI*. Oral presentation at ATSDR All Partners' Meeting, Atlanta, GA
- 2011 **Krajewski A**, Cody G, and Heiger-Bernays W. *Food Safety and Emergency Planning for Retail Food Establishments*. Poster presented at the National Environmental Health Association (NEHA), 75th Annual Educational Conference and Exhibition, Columbus, OH

Selected Awards & Honors

- 2015-2016 Provost's Award for Graduate Research, University of Illinois at Chicago
Principal Investigator and Grant Manager
- 2015 Honorable Mention, Student Poster Presentations, Environment Section,
American Public Health Association (APHA), 143rd Annual Meeting and
Expo, Chicago, IL

Student Organizations

- 2014-Present *Co-founder and Vice President*
Student Alliance for Public Health and the Environment (SAPHE)
University of Illinois at Chicago
- 2015-2016 *Graduate Assistant*
Sensor Technology Targeted Research Team
University of Illinois at Chicago