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A Large Cohort Linkage Study of Lead Exposure for Mortality and End Stage Renal Disease

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ABSTRACT

A Large Cohort Linkage Study of Lead Exposure for Mortality and End Stage Renal Disease

We studied the association of lead exposure with mortality and end-stage renal disease (ESRD) incidence in 58,000 subjects who were part of an occupational lead surveillance in 11 states and had blood lead levels (BLLs) recorded during the period 1982-2005. Subjects were divided into four groups, based on their highest BLL: <5 µg/dl, 5 to <25 µg/dl, 25 to <40 µg/dl, and 40+ µg/dl.

In the first study, we compared the lead-exposed cohort's mortality to the US population, and did internal comparisons of high lead groups compared to low. In the second study, we conducted similar analyses for ESRD incidence. In the third study, we used Cox regression to study risk of mortality after ESRD incidence by lead category.

In the first study, we found evidence of increased risk of lung and larynx cancer with higher lead exposure, with significant positive trends in lung cancer by increasing lead category (test for trend p=0.0001). The SMR for highest blood lead category was 1.2, increasing to 1.35 with 20 years latency. Positive trends were also seen for mortality due to heart disease and kidney disease. Data are limited by a lack of work history and smoking data, different follow-up time for different lead categories, and small numbers of deaths for some causes. In our second study, we found evidence for increased ESRD incidence for those in the highest BL category (51+µg/dl) in this cohort (standardized rate ratio for highest blood lead category, with 5 years latency, 1.59). In our third study, we found no association between blood lead level and survival after ESRD diagnosis.

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LIST OF ABBREVIATIONS

ABLES	Adult Blood Lead Epidemiology and Surveillance
ACE	Angiotensin converting enzyme
ACGIH	American Conference of Governmental Industrial Hygienists
BL	Blood Lead
BLL	Blood Lead Level
BMI	Body Mass Index
BP	Blood Pressure
CA	Cancer
CDC	Centers for Disease Control and Prevention
CHD	Coronary Heart Disease
CI	Confidence Interval (usually 95% unless mentioned otherwise)
CKD	Chronic Kidney Disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic Blood Pressure
dl	Deciliter
DNA	Deoxyribonucleic acid
DoB	Date of Birth
EPA	United States Environmental Protection Agency
ESRD	End Stage Renal Disease
FMD	Flow mediated dilatation
Hg	Mercury / hydrargyrum
HR	Hazard Ratio
IARC	International Agency for Cancer
IFN	Interferon
IL	Interleukin
IMT	Intima medial thickness
KXRF	K-shell X-ray fluorescence
LOD	Limit of Detection
mm	Millimeter
Mn	Manganese
NAAQS	National Ambient Air Quality Standards
NDI	National Death Index

NHANES	National Health and Nutrition Examination Survey
NHS	Nurses' Health Study
NIH	National Institutes of Health
NIOSH	National Institutes of Occupational Safety and Health
NLMS	National Longitudinal Mortality Study
NTP	National Toxicology Program
OR	Odds Ratio
OSHA	Occupational Safety and Health Administration
RBC	Red blood cells or erythrocytes
RR	Risk Ratio
SBP	Systolic Blood Pressure
SES	Socio-economic status
SMR	Standardized Mortality Ratio
TIA	Transient Ischaemic Attack (stroke <24 hours duration)
TNF	Tumor necrotic factor
US/USA	United States of America
USRDS	United States Renal Data System
VA	Veteran's Administration
µg	Micrograms
µmole	Micromole

CHAPTER 1: INTRODUCTION

Study Motivation

Lead is neurotoxic in children (Bryce-Smith 1972, Blackwood 1975, de la Burde *et al.* 1975, Valdes Bolanos 1975), and can cause acute poisoning in adults (White 1975, Agency for Toxic Substances and Disease Registry 2007, Garcia-Leston *et al.* 2010). However, it is less recognized that lead may also cause chronic health effects in adults. Adult chronic exposure to lead has been associated with multiple outcomes, including hypertension, cancers, heart disease, and non-malignant kidney disease among others; however, the evidence is not conclusive. With the US Environmental Protection Agency's (EPA) establishment of the permissible level of lead in the air (U.S. Environmental Protection Agency 1977), and reduction of lead use in commercially available products (particularly leaded gasoline), population lead exposure has largely been limited to those occupationally exposed. The National Institute of Occupational Safety and Health (NIOSH) have estimated that more than 3 million workers in the US are potentially exposed to lead at work (Rempel 1989, Staudinger *et al.* 1998). The current Occupational Safety and Health Administration (OSHA) standard calls for the removal of workers from exposure when their blood lead levels (BLL) are greater than 50 µg/dl, and stay removed until their BLLs decline below 40 µg/dl; although a number of authors have called for removal of workers from exposure when BLLs are 20 µg/dl or higher (Hu *et al.* 2007, Kosnett *et al.* 2007, Schwartz *et al.* 2007, Spivey 2007). These authors point out that the current lead standards were set in order to avoid acute symptoms of lead poisoning, but do not appear protective against chronic disease outcomes, as evidenced by studies in the last 10-15 years as discussed below.

Increasing evidence in the last decade links lead exposure, even at relatively low environmental levels, to blood pressure changes (Alghasham *et al.* 2011, Poreba *et al.* 2011c, Wells *et al.* 2011), heart disease (Saric 1981, Jain *et al.* 2007), cancer (Gwini *et al.* 2012, Ilychova *et al.* 2012, Wu *et al.* 2012), and kidney dysfunction (Saric 1981, Muntner *et al.* 2003, Navas-Acien *et al.* 2009). Both the International Agency for Cancer (IARC) and the National Toxicology Program (NTP) have recently declared lead to be a probable human carcinogen, primarily based on lung and stomach cancer studies, with brain and kidney cancer also being elevated in some studies. The weight of the evidence indicates that lead exposure increases blood pressure in adults, making both stroke and heart disease outcomes of interest. Very high lead exposure is known to cause non-malignant kidney disease, and there is increasing evidence that low-levels can do the same.

In 2010, ABLES reported 31,081 adults from 40 states had BLLs ≥10 μg/dl, of which 8,793 had BLLS ≥25 μg/dl and 1,388 had BLLs ≥40 μg/dl. To add perspective to these BLLs, the current geometric mean US adult blood lead level is <3 μg/dl (National Institute for Occupational Safety and Health 2012). Thus, lead exposure remains a national occupational health problem, and further work is needed to reduce lead exposures. As BLL data is often not available for many workers, due to inadequate reporting or testing, the actual rates of lead exposure or numbers exposed to lead might be much higher (National Institute for Occupational Safety and Health 2012). Since there is no identified blood lead level without harmful effects, further study is warranted to identify the health effects of lead exposure.

We studied the mortality and end-stage renal disease incidence in approximately 58,368 male participants who have been in NIOSH-sponsored blood lead surveillance programs in eleven states from 1987-2005. Subjects were divided into four groups of lead exposure, based on their highest blood lead level recorded during the study period. The four groups were: <5 μg/dl, 5 to <25 μg/dl, 25 to <40 μg/dl, and 40+ μg/dl. OSHA recommends that active workers have blood lead levels below 40 μg/dl, and American Conference of Governmental Industrial Hygienists (ACGIH) recommends that active workers have BLLs lower than 30 μg/dl. The outcomes of interest were death from cancer, stroke, heart disease, and non-malignant kidney disease, as well as the incidence of End Stage Renal Disease (ESRD). In external analyses, mortality and ESRD incidence rates of the cohort were compared to that of the US population. In addition, internal analyses compared those with higher blood lead levels to the group with lowest blood lead levels (<5 μg/dl). Among those diagnosed with ESRD, we evaluated the association of lead exposure in categories with survival, adjusting for known confounders of the association using Cox proportional hazards (PH) models. The population of interest has the advantage of having documented blood lead levels, and of being larger than any previously studied cohort with information on lead exposure.

Study Contribution

Both the International Agency for Cancer (IARC)(International Agency for Research on Cancer 2006) and the National Toxicology Program (NTP) (National Toxicology Program 2004) have recently declared lead to be a probable human carcinogen, primarily based on findings for lung and stomach cancer, with brain and

kidney cancer also being elevated in some studies. The weight of the evidence indicates that lead exposure increases blood pressure in adults, making both stroke and heart disease a key outcome of interest. High lead exposure is known to cause non-malignant kidney disease, but it is not known if lower levels lead to this outcome. However, current epidemiological data is inconclusive and more research is needed to determine the association of lead exposure with cancers (brain and kidney), ischaemic heart disease and stroke. The present study sought to overcome issues with previous occupational cohort studies, such as small sample size and reliance solely on mortality data, by examining a large cohort with documented blood lead levels and gathering information on morbidity (incident ESRD) in addition to mortality. Further, our study population included a large number of subjects with BLLs ≥40 μg/dl, the maximum level that OSHA considers acceptable and safe for occupationally exposed workers.

For the present study, we obtained data on subjects participating in the ABLES program from 11 states. The state ABLES program collects information on blood lead tests from laboratories throughout each state, as part of an occupational lead surveillance program sponsored by the National Institute for Occupational Safety and Health (NIOSH) (details below). Thus, the present study has provided a model of pooling state based surveillance data, as has been called for by a 2001 panel of environmental and public health experts (the Pew Environmental Health Commission) (Litt *et al.* 2004). In response to the Pew Commission, the Centers for Disease Control (CDC) set up an initiative to promote the pooling of state environmental data, called the Environmental Public Health Tracking Program (http://www.cdc.gov/nceh/tracking/) (McGeehin *et al.*

2004). Our study also addresses a recent call for further research by IARC to study the role of lead exposure on cancer mortality, to help resolve ambiguities in the existing literature (Ward *et al.* 2010).

Study Objectives

The overarching objective of this dissertation was to investigate the health effects of chronic lead exposure on all-cause mortality, cause-specific mortality, incident ESRD and survival after diagnosis of ESRD.

In the first study, we examined the effect of lead exposure on mortality by comparing the all-cause and cause-specific mortality patterns of ABLES participants by BLL category versus US mortality rates in external comparisons. In internal comparisons, we compared the mortality patterns of moderately exposed (5≤BLL<25μg/dl), intermediately exposed (25≤BLL<40 μg/dl), and highly exposed (BLL≥40 μg/dl) subjects with those of subjects with low BLL (<5μg/dl).

For our second study, we divided the highest BLL category (≥40 μg/dl) based on its median (51μg/dl) into 2 categories. Using these 5 categories, we compared the incidence rates of ESRD by BLL with that of the US population (external comparison), and the incidence of ESRD of participants in the upper four BLL categories to those in the lowest BLL category (<5μg/dl) (internal comparison). Since race is a potential confounder in these analyses and was missing for 69% of our cohort, we also examined this association in the subset of male subjects with known race and in the full cohort of male subjects after imputing race (for the 69% of subjects with missing race information)

by using established imputation techniques (PROC MI and Monte Carlo estimation).

For our third study, using 5 categories of lead exposure (<5μg/dl, 5-<25μg/dl, 25-<40μg/dl, 40-<50 μg/dl and ≥50μg/dl), we evaluated the effect of BLL on the survival patterns among ESRD patients using a Cox proportional hazards (PH) model, adjusted for body mass index (BMI), glomerular filtration rate (eGFR), transplantation status, race, ethnicity and other potential confounders.

CHAPTER 2. LITERATURE REVIEW

Lead is neurotoxic in children (Watters *et al.* 1967, Chisolm 1970, Paci *et al.* 1970, Bryce-Smith 1972, 1975, Blackwood 1975, de la Burde *et al.* 1975, Franco *et al.* 1975, Pueschel *et al.* 1975, Puschel 1975, Shellshear *et al.* 1975, Valdes Bolanos 1975, Zarkovsky 1975), and can cause acute poisoning in adults (Alexander 1975, Blumer 1975, CDC 1975, Chisolm *et al.* 1975, Julia *et al.* 1975, Lepow *et al.* 1975, Sanai *et al.* 1975, Wedeen *et al.* 1975, White 1975, Agency for Toxic Substances and Disease Registry 2007, Garcia-Leston *et al.* 2010). These research findings led the US EPA to establish the National Ambient Air Quality Standard (NAAQS) for lead to meet requirements of the U.S. Clean Air Act (U.S. Environmental Protection Agency 2006). In the mid-1970's, lead (Pb) was first listed as a criteria air pollutant and relevant scientific information led to EPA establishing a 1.5 $\mu g/m^3$ (maximum quarterly calendar average) for regulation of lead in air (U.S. Environmental Protection Agency 1977). However, it is not clear if lead is likely to cause chronic health effects in adults.

With the establishment of air pollution limits and subsequent reduction of lead use in commercially available products, especially gasoline, the major populations exposed to lead have been limited to occupational cohorts working in industries associated with lead and accidental lead exposure and poisoning. Thus, the chronic health effects in adults can perhaps be best studied in occupational cohorts. Multiple studies have looked at occupational cohorts (Cooper *et al.* 1985, Fanning 1988, Steenland *et al.* 1990, Steenland *et al.* 1992, Payton *et al.* 1994, Fu *et al.* 1995, Gerhardsson *et al.* 1995, Cocco *et al.* 1997, Fischbein 1998, Lustberg *et al.* 2002, Nawrot *et al.* 2002, Checkoway H 2004, Ekong *et*

al. 2006, Radican *et al.* 2006). Spivey (2007) (Spivey 2007) reviewed the varied effects of adult lead exposure (including hypertension, kidney disease, neurotoxicity, and brain cancer). The author stresses that most elevated blood lead levels today are due to occupational exposure, and he questions whether the current occupational standards are adequate. In a mini-monograph on lead, which recently appeared in Environmental Health Perspectives and was inspired by the ABLES program, both Hu *et al.* (2007) (Hu *et al.* 2007) and Schwartz *et al.* (2007) (Schwartz *et al.* 2007) and Kosnett *et al.* (2007) (Kosnett *et al.* 2007) call for removal of workers from exposure when blood levels reach 20 µg/dl, in contrast to current OSHA requirements removing workers from exposure only when their blood lead levels are above 40 µg/dl. These authors point out that current lead standards were set in order to avoid acute symptoms of lead poisoning, but do not appear protective against chronic disease outcomes, as evidenced by studies in the last 10-15 years. Thus, further research is needed to establish blood levels of lead that are associated with adverse health effects (Rosin 2009), to ensure better regulation of occupational exposure levels.

In the next few sections, we looked at the current literature regarding the pathophysiology of lead, and proposed mechanisms of lead toxicity, following acute and chronic exposure. Further, we reviewed current scientific literature on the association of lead exposure and subsequent development of diseases, focusing on the aforementioned diseases of interest.

Pathophysiology

Lead has been demonstrated to be toxic in most forms and routes of entry. Major routes of exposure to lead and its compounds include inhalation or ingestion of contaminated water, food, and through air, and soil (Rabinowitz *et al.* 1974, Rabinowitz *et al.* 1976, DeMichele 1984). Occupational exposure is one of the common causes of lead poisoning, with the National Institute of Occupational Safety and Health (NIOSH) estimating that more than 3 million workers in the US are potentially exposed to lead at work (Rempel 1989, Staudinger *et al.* 1998). In the present study, exposure to lead in the air is the major route of exposure. Animal studies show that certain substances bind with lead and increase its solubility, thereby increasing lead absorption when ingested and inhaled (the major routes of lead entry into the body) (DeMichele 1984).

The duodenum is the primary site of lead absorption. However, unlike other nutrients, there is no negative feedback mechanism to regulate lead absorption. Thus, the total body lead content does not limit or regulate lead absorption. Lead absorbed from the intestine occurs by both active transport and passive diffusion. In our present study, the major route of lead exposure is inhalation. However, the mechanism for lung absorption is unknown. If the particle size is < 1 μm, such as in lead fumes, then absorption is high (>90%) (Rabinowitz 1998). Particles > 2.5 μm diameter containing lead get deposited in the ciliated membranes of the nasopharynx and respiratory airways. They are transported, from these sites by *mucociliary lift* mechanism, to the gastrointestinal tract and absorbed. Once lead enters the bloodstream, lead is transported predominantly by being bound to red blood cell (RBC) proteins (Barltrop *et al.* 1972, Rabinowitz *et al.* 1974, Barltrop *et al.* 1975, Rabinowitz *et al.* 1976, Rabinowitz *et al.* 1977, Simons 1984, Simons 1988, Rabinowitz 1991, Church *et al.* 1993a, Church *et al.* 1993b, O'Flaherty 1993, Bergdahl *et*

al. 1997a, Bergdahl *et al.* 1997b, Bergdahl *et al.* 1997c, Bergdahl *et al.* 1997d). Lead also binds to other proteins, especially to thiol and carboxyl groups of proteins, and mimics calcium in different biologic pathways (Rabinowitz *et al.* 1973a, Rabinowitz *et al.* 1973b, Goldstein *et al.* 1983, Rabinowitz 1991, Kern *et al.* 2000a, Kern *et al.* 2000b). Thus, lead is circulated widely and may be found in all tissues and organs. Lead also crosses the placental barrier (leading to toxicity of the fetus) and blood-brain barrier leading to neurotoxicity (Hu 1998). Bile is an important route of lead excretion in the gut. However, a large percentage of the lead excreted would be re-absorbed, as duodenum is also the primary site of absorption, thereby leading to decreased lead loss and maintenance of lead concentration in the body (DeMichele 1984). Studies done to evaluate the metabolism of lead show that after ingestion, lead is distributed in blood compartment, with an average half-life of 35 days, soft tissues, including hair, nails, sweat, and salivary, gastric, pancreatic, and biliary secretions, with an average half-life of 40 days and bones. Bones differ in their rates of lead turnover, and lead in bone has a very long half-life (Rabinowitz *et al.* 1974). Lead has an affinity for bones, with more than 90% of lead absorbed being deposited in bones in a relatively inert form, by replacing calcium (DeMichele 1984). Lead in teeth, hair, nails and bones are tightly bound and not readily available. In children, about 70% is deposited in bones, accounting for the more severe health effects (Barbosa *et al.* 2005). Half-life of lead deposited in bone is estimated to be 20-30 years (Patrick 2006). Thus, after an acute exposure is over, the person could still be exposed with small amounts being released into the circulation over a long period of time, due to bone re-modeling (Barbosa *et al.* 2005). If lead exposure takes place over time, clearance is at a much slower rate due to release of bone lead (Hu *et al.* 2007). The

main route of excretion of lead is through urine, however, lead exposure may cause kidney damage, which decreases urine output and results in further increases in lead levels in the body. Other routes of excretion include faeces and small amounts through hair, nails, and sweat.

Review of Epidemiologic Literature by Outcome

1. Cancer

The epidemiological data for lead exposure and cancer consistently show associations with stomach cancer, while some studies (but not all) show increased risk of lung, kidney and brain cancer. Detailed reviews of lead carcinogenicity may be found in the recent monographs by the International Agency for Research on Cancer (International Agency for Research on Cancer 2006) and the National Toxicology Program (National Toxicology Program 2004), and meta-analyses for cancer have been written by Steenland and Boffeta (Steenland *et al.* 2000), and Fu and Boffetta (Fu *et al.* 1995). Inorganic lead causes cancer in rats and mice when administered orally or via injection, in either soluble (lead acetate) or insoluble lead forms (lead phosphate and chromate) (Tiffany-Castiglioni *et al.* 1986, Tiffany-Castiglioni *et al.* 1988, Tiffany-Castiglioni *et al.* 1989, Tiffany-Castiglioni 1993, Tonner *et al.* 1997, Qian *et al.* 1999, Chen *et al.* 2002). Kidney tumors (Hiasa *et al.* 1983, Koller *et al.* 1985, Tiffany-Castiglioni *et al.* 1986, Short *et al.* 1987) are most frequently seen, but brain (Tiffany-Castiglioni *et al.* 1988, Tiffany-Castiglioni *et al.* 1989, Tiffany-Castiglioni 1993, Tonner *et al.* 1997, Qian *et al.* 1999), blood (Tiffany-Castiglioni *et al.* 1986), and lung tumors (Green *et al.* 1997) have also been reported. Human cytogenetic studies indicate damage to chromosomes or DNA, but studies are not consistent. The mechanism by which lead causes cancer is not understood, but it is

thought to involve DNA synthesis and repair, by interacting with DNA binding or tumor suppressor proteins or by generating reactive oxygen species (ROS) rather than direct genetic damage (Lu *et al.* 2002, McNeill *et al.* 2007).

In 2004, both IARC(International Agency for Research on Cancer 2006) and NTP(National Toxicology Program 2004) concluded that lead was a probable human carcinogen, based primarily on lung and stomach cancer studies, with some suggestion of an effect for kidney and brain cancer (Baker *et al.* 1980, Kang *et al.* 1980, Lilis 1981, Fu *et al.* 1995, Kurt 1995, Lundstrom *et al.* 1997, Cocco *et al.* 1998, Siddiqui *et al.* 2002, van Wijngaarden *et al.* 2006, Rousseau *et al.* 2007, McElroy *et al.* 2008, Alatise *et al.* 2010, Wu *et al.* 2012). The most informative human epidemiology comes from seven cohort studies of occupationally exposed cohorts (Fanning 1988, Steenland *et al.* 1992, Anttila *et al.* 1995, Gerhardsson *et al.* 1995, Lundstrom *et al.* 1997, Wong *et al.* 2000, Carta *et al.* 2005). Additional information comes from four mortality follow-up studies of the NHANES II and NHANES III populations (Jemal *et al.* 2002, Lustberg *et al.* 2002, Menke *et al.* 2006, Schober *et al.* 2006). The NHANES II and NHANES III populations are representative samples of the US population from the late 1970s and the late 1980s respectively, for which blood lead measurements are available.

A meta-analysis of the seven occupational cohorts (Steenland *et al.* 2000), with 30,000 workers, found a combined lung cancer rate ratio of 1.30 (95% CI 1.15-1.46, 675 deaths) and a combined stomach cancer rate ratio of 1.34 (95% CI 1.04-1.73, 181 deaths). There was little evidence for kidney cancer (RR 1.10, 95% CI 0.72-1.42, 40 deaths), or brain cancer (RR 1.06, 95% CI 0.8-1.40, 69 deaths) in relation to lead exposure, although for both these outcomes some individual studies reported significantly elevated RRs. The

lung cancer finding was diminished when the authors excluded one study in which there was co-concomitant exposure to arsenic. Mean blood lead levels in six of these cohorts, typically measured in the 1950s-1970s, ranged from 40 µg/dl to 80 µg/dl, while for one study the mean level was 26 µg/dl. Wong *et al*. (2000) (Wong *et al.* 2000), in a study of 4,518 lead battery plant workers and 2,300 lead smelter workers, found significant associations between occupational lead exposure and mortality due to stomach cancer (SMR 1.47, 95% CI: 1.12, 1.89), lung cancer (SMR 1.16, 95% CI: 1.04,1.29), and cancer of endocrine glands (SMR 3.08, 95% CI: 1.33, 6.07). They also found associations with cancer of the kidneys and brain, but these were not significant.

A more recent study of brain cancer, based on a large number of US death certificates by van Wijngaarden *et al*. (2006) (van Wijngaarden *et al.* 2006), using the National Longitudinal Mortality Study (NLMS) data with 317,968 participants, found a two-fold significant increased risk (HR 2.3, 95% CI: 1.3, 4.2 for those in occupations with highest probability of exposure and highest intensity of exposure. A study using the New Jersey lead ABLES population with 3,192 participants found non-significant elevations in cancers of the stomach, breast, larynx, intrahepatic bile duct, and chronic myeloid leukemia (Lam *et al.* 2007).

Regarding the general population studies, Jemal *et al*.(2002) (Jemal *et al.* 2002) studied 3,592 white US participants in NHANES II (1976-1980) who had known blood lead levels at baseline, with follow-up through 1992 for mortality. Authors adjusted for age, alcohol, and smoking. Median blood lead levels were 12 µg/dl. Relative risk of all cancers was elevated in the top quartile vs. the bottom for both men (RR 2.0, 95% CI 0.6-6.5, 47 deaths in top quartile) and women (RR 1.6, 95% CI 0.8-3.3, 23 deaths in top

quartile); quartile trend analysis for men and women combined for all cancer showed a positive trend which was not statistically significant at the 0.05 level (p=0.16). The RR for lung cancer for those above the median BLL vs. those below was 1.5 (95% CI 0.7-2.9, 71 deaths), and the corresponding RR for stomach cancer was 2.4 (95% CI 0.3-19.1, 5 deaths). This study was limited by the small number of deaths. Lustberg and Silbergeld (2002) (Lustberg *et al.* 2002) studied the same population (again with follow-up through 1992), but included nonwhites and controlled for more confounders (e.g., obesity), but excluded persons with BLLs>30 µg/dl, on the basis that these would have had occupational exposure resulting in 4,292 participants aged 30 to 74 years. Using those with <10 µg/dl as the referent, they found an excess for all-cancer (cancers due to all causes) in those with BLLs from 10-20 µg/dl (1.5, 95% CI 0.9-02.5) and for those with 20-30 µg/dl (RR 1.7, 95% CI 1.0-2.8).

There are now similar findings for the NHANES III population. Schober *et al*. (2006) (Schober *et al.* 2006) followed 9,757 participants who were of ages 40 or older at baseline, for an average of 10 years. They categorized subjects into <5 µg/dl, 5-9 µg/dl, and 10+ µg/dl, and found a significant increasing trend (p<0.001) in all-cancer deaths (n=543), with RRs of 1.00, 1.44 (1.12-1.86), and 1.69 (1.14-2.52) respectively. The authors adjusted for a variety of risk factors, including SES and smoking. There was no breakdown by individual cancers. Menke *et al*. (2006) (Menke *et al.* 2006) studied the same population aged 20 and over with 13,946 adult participants (408 cancer deaths), but used different cut-points (<1.9 µg/dl, 1.9-3.6, µg/dl, >3.6 µg/dl). They found RRs of 1.00, 0.72 (.46-1.12) and 1.10 (0.82-1.47) by increasing exposure, after adjusting for covariates, with a p-value of 0.10 for trend. Again no data were presented on specific

cancers. The different cut-points and inclusion of younger subjects presumably accounted for the lack of a cancer trend in Menke *et al*. (2006) (Menke *et al.* 2006) vs. Schober *et al*. (2006) (Schober *et al.* 2006). More recently, studies in other countries have shown a higher risk of all-cancers among those highly exposed to lead (Chang *et al.* 2009, Wu *et al.* 2012). Areas with higher gas stations density, a proxy for air pollutant and lead exposure, were found to have higher mortality associated with various cancers in Taiwan (Liu *et al.* 2008a, Liu *et al.* 2008b, Weng *et al.* 2008, Chang *et al.* 2009, Liu *et al.* 2009, Tsai *et al.* 2009, Weng *et al.* 2009, Chiu *et al.* 2011, Wu *et al.* 2011, Wu *et al.* 2012). Two studies, one by Weisskopf *et al*. (2009) (Weisskopf *et al.* 2009) among 868 male veterans who were part of the Normative Aging Study, and Khalil *et al*. (2009) (Khalil *et al.* 2009) in a prospective cohort study of 533 women aged 65-87 years enrolled in the US Study of Osteoporotic Fractures, did not find an association between lead and cancer. A study on lead by Ilychova *et al*. (2012) (Ilychova *et al.* 2012), with 1,423 male and 3,102 female workers in the printing industry, found mortality from all cancers combined was lower than that of the general population in Moscow, probably due to healthy worker effect. However, in internal comparisons, mortality from kidney (SMR 2.12, 95% CI 1.10 to 4.07) and pancreatic cancers (SMR 2.32, 95% CI 1.46 to 3.68) increased by almost twofold in the highest tertile compared to the lowest tertile. Another study of 4,114 male lead workers found an increased risk of death (SMR 1.11, 95% CI: 1.01, 1.23), liver cancer (SIR 2.17, 95% CI: 1.03, 4.54) and esophageal cancer (SIR 2.4, 95% CI: 1.29, 4.47) (Gwini *et al.* 2012). Although they found slight elevations in rate ratios for lung, stomach and brain cancer, these were not statistically significant at the 0.05 level.

In summary, the data are suggestive of an excess risk of lung cancer and stomach cancer due to lead exposure, with some studies also suggesting a risk for kidney and brain cancer. The population-based studies are intriguing in showing positive trends in all cancer mortality, based on a single blood measurement, but show only sparse evidence regarding risks for specific cancers.

2. Blood Pressure

Animal models have shown statistically significant increases in blood pressure among those exposed to lead (Fiorim *et al.* 2011). Even low dose lead exposure has been associated with such increases (Reza *et al.* 2008, Fiorim *et al.* 2011). The increases in systolic pressure have been attributed to the effect of lead on increasing angiotensin II levels due to ACE activation (Simoes *et al.* 2011). Lead exposure has been associated with decreased brachial artery flow-mediated dilation (FMD) due to increased arterial stiffness, and increased carotid intima-media thickness (Poreba *et al.* 2010a, Poreba *et al.* 2010c, Poreba *et al.* 2011c). Studies found significantly lower FMD (Poreba *et al.* 2010c) and significantly higher (even among normotensives) mean IMT values (Poreba *et al.* 2010a, Poreba *et al.* 2011c) among lead exposed workers as compared to the control group.

There have been a large number of studies among humans of blood pressure and lead exposure. The vast majority have been cross-sectional, with a handful of longitudinal studies. A meta-analysis of the lead-blood pressure studies has been conducted by Nawrot *et al*. (2002) (Nawrot *et al.* 2002). These authors included 31 studies (only 4 prospective in design) with sufficient details to analyze dose-response. Most of these

studies were based on the general population, although seven were populations occupationally exposed to lead. All studies controlled for age, and most took into account additional confounding factors such as BMI, alcohol use, and blood pressure medication. While not all studies were consistent, most showed a positive association between blood lead levels and blood pressure. A doubling of blood lead (using the mean as baseline in studies using untransformed lead in linear models) was associated with 1.0 mm rise in systolic pressure (95% CI 0.5-1.4), and a 0.6 mm Hg increase in diastolic pressure (95% CI 0.4-0.8). Overall, there was no significant heterogeneity between studies, nor between those studies which used the log of blood lead vs. untransformed blood lead, nor between studies of men and women. In those studies in which whites and blacks were analyzed separately, the lead-blood pressure association was stronger in blacks, but the difference was not statistically significant.

An important longitudinal study of bone lead and hypertension in the general population found that those in the highest quintile of bone lead had a 1.7 fold significant excess risk of developing hypertension over the 8 year follow-up period, after adjustment for confounders (Cheng *et al.* 2001). The 833 subjects in this study were men in the 1990s, with a mean blood lead of 6 µg/dl (VA Normative Aging Study). Higher blood lead also predicted higher blood pressure, but less so than bone lead. Bone lead is a good indicator of cumulative exposure over time, unlike blood lead, which reflects recent exposure.

The general scientific view, based on a number of retrospective and prospective studies, is that lead exposure causes an elevation of blood pressure while prolonged exposure even to low levels of lead may lead to arterial hypertension (Sharp *et al.* 1987,

Wiecek *et al.* 1987, Nowack *et al.* 1992, Batuman 1993, Hertz-Picciotto *et al.* 1993, Staessen *et al.* 1994a, Staessen *et al.* 1994b, Schwartz 1995, Solomenchuk 1995, Staessen 1995, Staessen *et al.* 1995, Staessen *et al.* 1996, Korbakova *et al.* 2001, Den Hond *et al.* 2002, Nawrot *et al.* 2002, Akhmetzianova *et al.* 2006, Agency for Toxic Substances and Disease Registry 2007, Popov *et al.* 2007, Skoczynska *et al.* 2007, Doroszko *et al.* 2008, Poreba *et al.* 2011a). More recently, Poreba *et al*. (2011) (Poreba *et al.* 2011a) found multiple studies that supported the link between lead exposure and subsequent development of arterial hypertension. They found that employees chronically exposed to lead with an average period of employment of 25 years had a mean blood pressure increase of 11 mm of Hg on average (SBP and DBP increase was 14 mm of Hg and 8 mm of Hg above normal), as compared to non-exposed people (Poreba *et al.* 2010b, Poreba *et al.* 2011b). Poreba *et al*. (2010) also found concurrent exposure to lead and other heavy metals, like cadmium, were associated with higher arterial blood pressure values (Poreba *et al.* 2010b), leading us to believe that there might be interactions of lead exposure with other heavy metal exposure in development of hypertension. However, the authors also found an independent effect of lead exposure on future hypertension. Poreba *et al*. (2010) found positive linear correlations between pulse pressure and blood lead level. Higher blood lead levels were found to be an independent risk factor for increases in pulse pressure (Poreba *et al.* 2010b). According to Wells *et al*. (2011) (Wells *et al.* 2011), in a study of 285 women, even low dose lead exposure in pregnant women was associated significantly with elevation in blood pressure, 6.87 mmHg (95% CI: 1.51-12.21 mmHg), increase in systolic and a 4.40 mmHg (95% CI: 0.21-8.59 mmHg), increase in diastolic blood pressure, after adjustment for confounders.

3. Stroke (Cerebrovascular Disease)

The increase in blood pressure with increased blood lead in human studies (described above) was modest, but nonetheless might be expected to lead to increased stroke in exposed populations. Increased blood pressure is a uniquely strong risk factor for stroke. It is a stronger risk factor for stroke, than for example coronary heart disease (CHD). Framingham data indicates that the relative risk of stroke and transient ischemic attack (TIA) for those with diastolic blood pressure (DBP) of 105-60 vs. 75-84 was 8.0, while the same RR for CHD was 2.7 (Cook *et al.* 1995). It is also the main risk factor for stroke, while CHD has a number of other important risk factors, including cholesterol and smoking. A meta-analysis of treatment effects in five clinical trials of hypertensive drugs showed a reduction of stroke of 34% vs. a reduction of CHD of 19% (NIH, 1997), a further indication of the importance of blood pressure for stroke vs. heart disease.

There is rather limited information on stroke due to lead exposure. Most information comes from the seven key occupational cohort studies cited above in the discussion about cancer. Of these, five have information on stroke, and of these, four show some indication of an excess for exposed workers, or sub-sets of exposed workers, compared with low or non-exposed populations. Fanning (1998) (Fanning 1988), in a case-control study of 867 deaths among lead exposed, compared to 1,206 deaths among low or unexposed deaths, reported an odds ratio of 1.24, of borderline statistical significance, for high exposed workers vs. those with low or no exposure. However, when he compared workers with high lead exposure vs. low lead exposure by calendar time, he found that there was an odds ratio of approximately 2.0 for high lead vs. low lead before 1965, when lead exposures were thought to have been higher, which were

significant at the 0.05 level (46 deaths in the high lead group). Further suggestive evidence was found by Steenland *et al*. (1992) (Steenland *et al.* 1992) in a study of 1,990 smelter workers which compared US smelter workers with the US population and found no overall excess of stroke (ICD 430-438) (SMR 1.05 for all workers and also for high lead workers), but did find an elevation for workers with 20 or more years employment (SMR 1.41, 95% CI 0.95-1.85), 26 deaths). Cocco *et al*. (1997) (Cocco *et al.* 1997) studied 1,388 Italian smelter workers and found an SMR of 1.22 (95% CI 0.98-1.49, 93 deaths) using regional comparison rates. Gerhardsson *et al*. (1995) (Gerhardsson *et al.* 1995) studied 664 male smelter workers and found a SMR for stroke of 1.72 (95% CI 1.20-2.42. 34 deaths), increasing to 1.81 (95 % CI 1.22-2.65) for those employed before 1969, when exposures were higher. On the other hand, Lundstrom *et al*. (1997) (Lundstrom *et al.* 1997), in their study of 1,992 smelter workers, found no excess for stroke in either the total cohort or the high exposed sub-cohort (SMRs of 0.8 and 0.9, respectively).

More recent evidence on stroke comes from the NHANES III population. Menke *et al*. (2006) (Menke *et al.* 2006) studied 13,946 NHANES III participants who were studied at baseline in the late 1980s, at which time their blood lead was measured. These authors categorized their data into three groups, <1.9 µg/dl, 1.9-3.6, µg/dl, and >3.6 µg/dl, based on a single blood lead measurement at baseline. After a 10 year follow-up, they found RRs of 1.00, 2.19 (0.87-5.53) and 2.51 (1.20-5.26), with a p-value of 0.02 for trend, based on a total of 141 stroke deaths. The corresponding RRs for myocardial infarction (MI) were 1.00, 1.02 (0.55-1.89) and 1.89 (1.04-3.43), with a p-value for trend of 0.01, based on 367 MI deaths. Schober *et al*. (2006) (Schober *et al.* 2006), with 9,757

participants, also found a positive trend in the same population for a broad category of cardiovascular disease, which included MI and stroke, RRs of 1.0, 1.20 (0l93-1.55) and 1.55 (1.16-2.07), p-value <0.01 (1189 deaths), using cut-points of <5 µg/dl, 5-9 µg/dl, and 10+ µg/dl, respectively. A recent study of Russian lead workers with 1,423 male and 3,102 female workers, in spite of large number of deaths due to lead exposure, did not find a significant association between lead exposure and cerebrovascular disease (SMR 0.65, 95% CI 0.49 to 0.85) (Ilychova *et al.* 2012). In a study of 4,114 male lead workers by Gwini *et al*. (2012) (Gwini *et al.* 2012), the risk of stroke was found to be not significant (SMR 1.25, 95%CI: 0.84, 1.86).

In summary, the epidemiological data on the association of lead exposure and cerebrovascular disease is not conclusive, and more research is needed.

4. Cardiovascular or Heart Disease

i. Mechanisms: A cardiovascular disease (CVD) risk factor is *any measurable trait that may be linked to an increased probability of developing a future cardiovascular disease (Smith et al. 2004).* CVD risk factors can be classified into controllable risk factors [e.g., diet, smoking, physical activity, hypertension, elevated levels of total and LDL cholesterols, low level of HDL cholesterol, increased blood sugar levels (diabetes), obesity, inflammatory and prothrombotic factors] and uncontrollable risk factors (e.g., advanced age, male gender and genetic predisposition) (De Backer *et al.* 2003a, De Backer *et al.* 2003b, De Backer *et al.* 2004a, De Backer *et al.* 2004b). Lead exposure has been linked with multiple CVD risk factors in the toxicological and epidemiologic literature.

Multiple studies show an association between lead exposure and subsequent hypertension (vide supra). Lead exposure may cause endothelial dysfunction through a variety of mechanisms in both animal and human models (Gonick *et al.* 1997, Wagner *et al.* 1997, Vaziri *et al.* 1999a, Vaziri *et al.* 1999b, Skoczynska *et al.* 2000, Marques *et al.* 2001, Skoczyńska A. 2002, Stojek *et al.* 2003, Vaziri *et al.* 2003, Carmignani *et al.* 2004, Poreba *et al.* 2004, Zhan *et al.* 2004, Farmand *et al.* 2005, Zawadzki *et al.* 2006, Vaziri *et al.* 2007, Jomova *et al.* 2011, Poreba *et al.* 2011a). Lead exposure has been linked with novel CVD risk factors -elevated levels of homocysteine (Poreba *et al.* 2005, Schafer *et al.* 2005, Chia *et al.* 2007), C-reactive protein (CRP) and pro-inflammatory interleukins, decreases in blood interleukin 1β (IL-1β), interleukin 6 (IL-6) (Kaminska *et al.* 1998) and interferon-γ (IFN-γ) concentrations (Yucesoy *et al.* 1997), changes in tumor necrosis factor-α (TNF-α), and cytokine levels both in population and experimental studies (Hrycek *et al.* 1996, Yucesoy *et al.* 1997, Skoczynska *et al.* 2002, Di Lorenzo *et al.* 2007, Valentino *et al.* 2007). Lead causes target cells to release pro-inflammatory mediators and chemotactic and pro-coagulative factors mediated through increased production of ROS and interfering with anti-oxidative enzyme activity. Studies have demonstrated an inconsistent association of lead exposure with tachycardia, (Sroczynski *et al.* 1990, R. Poręba 2009), bradycardia and shortening of P-Q interval (Kosmider *et al.* 1961), rhythm disorders, prolonged P wave, QRS complex, and QT interval, and denivelations of ST segment (Stozinic *et al.* 1980, Saric 1981, Kirkby *et al.* 1985, Kromhout *et al.* 1985, Sroczynski *et al.* 1985, Shcherbak 1988, Sroczynski *et al.* 1990, Gatagonova 1995a, Gatagonova 1995c, Gatagonova 1995b, Cheng *et al.* 1998, Eum *et al.* 2011). Poreba *et al.* (2011) (Poreba *et al.* 2011d) found people occupationally exposed to lead showed heart

rhythm disorders, atrio-ventricular and intra-ventricular conduction disorders more often. Lead exposure has been associated with subsequent development of heart rate variability (HRV) (Murata *et al.* 1991, Teruya *et al.* 1991, Gennart *et al.* 1992, Murata *et al.* 1995, Ishida *et al.* 1996, Niu *et al.* 1998, Araki *et al.* 2000, Bockelmann *et al.* 2002, Andrzejak *et al.* 2004, Gajek *et al.* 2004, Jhun *et al.* 2005, Muzi *et al.* 2005), due to decreased parasympathetic tone (Murata *et al.* 1991, Murata *et al.* 1995, Andrzejak *et al.* 2004, Gajek *et al.* 2004, Poreba *et al.* 2011d). However, Gajek *et al*. (2004) (Gajek *et al.* 2004) were unable to confirm these findings in their study on occupationally exposed lead workers with blood lead levels below 500 µg/L. These mechanisms by which ROS may lead to CVD were seen in studies on atherosclerosis (Singh *et al.* 2006, Schleicher *et al.* 2007, Bonomini *et al.* 2008, Kondo *et al.* 2009, Victor *et al.* 2009a, Victor *et al.* 2009b, Chang *et al.* 2010, Hulsmans *et al.* 2010, Vaidya *et al.* 2011, Rosenson *et al.* 2012), primary arterial (Touyz *et al.* 2004, Touyz *et al.* 2011), reno-vascular (Friedman 2002, Higashi *et al.* 2002, Matz 2002, Ritter *et al.* 2002, Ziegler *et al.* 2002) and malignant hypertension (Lip *et al.* 2001, Lip *et al.* 2002), ischaemic heart disease (Gutteridge *et al.* 2000), myocardial infarction (Gutteridge *et al.* 2000), left ventricular re-modeling (Murdoch *et al.* 2006b, Sirker *et al.* 2007, Takimoto *et al.* 2007, Zhang *et al.* 2007, Hori *et al.* 2009, Kassiri *et al.* 2009, Krishnamurthy *et al.* 2009, Sun 2009, Takenaka *et al.* 2009, Tsutsui *et al.* 2009, Nabeebaccus *et al.* 2011), heart failure (both diastolic and systolic) (Cai *et al.* 2000, Lopez Farre *et al.* 2001, Tsutsui 2001, Sorescu *et al.* 2002, Byrne *et al.* 2003, Foster *et al.* 2003, Tsutsui 2003, Foster *et al.* 2004, Ungvari *et al.* 2005, Foster *et al.* 2006, Kinugawa *et al.* 2006, Murdoch *et al.* 2006a, Tsutsui 2006, Tsutsui *et al.* 2006, Seddon *et al.* 2007, Tsutsui *et al.* 2008, Benhar *et al.* 2009, Foster *et*

al. 2009, Kelleher *et al.* 2011, Kohlhaas *et al.* 2011, Tsutsui *et al.* 2011, Anand *et al.* 2012, Beigi *et al.* 2012, Qian *et al.* 2012), post-operative arrhythmias (Auer *et al.* 2005, Oral 2008, Van Wagoner 2008, Anselmi *et al.* 2009, McCarty 2010) and sudden cardiac death (White *et al.* 1987).

The American Heart Association has laid down criteria for regulation of blood pressure, so that blood pressure control will retard the development of or prevent the onset of heart disease (Rosendorff 2007). Poreba *et al*. (2010) found increases in pulse pressure were the main factor associated with increased frequency and severity of cardiovascular complications among patients occupationally exposed to lead. Markers of lead poisoning, especially higher levels of zinc protoporphyrin, were found to be an independent risk factor of left ventricular hypertrophy among patients with lead exposure and pre-existent hypertension (Poreba *et al.* 2010b).

ii. Epidemiologic studies: In a review of articles looking at lead and risk of CVD, Navas-Acien *et al*. (2007) (Navas-Acien *et al.* 2007) looked at 30 articles with clinical cardiovascular end points and 32 articles with intermediate cardiovascular end points. Overall, they found there was insufficient epidemiological data to make conclusive statements regarding the effects of lead exposure and CVD. Epidemiologic studies of lead exposure can be divided into two distinct types-those done in occupational cohorts and those in the general population.

a. Occupational studies: A recent study with 1,423 male and 3,102 female Russian lead workers (mostly printers), in spite of large number of deaths due to lead exposure, did not find a significant association between lead exposure and ischaemic heart disease (SMR 0.75, 95% CI 0.60 to 0.93), when compared to the

general population, but significant association in internal comparisons (SMR1.29, 95% CI 1.08 to 1.56) but only among male workers (Ilychova *et al.* 2012). In a study of lead workers (N= 1,990), Steenland *et al*. (1992) found a non-significant decrease in ischaemic heart disease (n=320, SMR 0.94, 95% CI: 0.84, 1.05), probably due to healthy worker effect (Steenland *et al.* 1992). Gerhardsson *et al*. (1995) (Gerhardsson *et al.* 1995), in a study of 664 male lead battery workers, found an increased mortality from ischaemic heart diseases (SMR 1.72; 95% CI 1.20-2.42). Other occupational studies mentioned above did not report cardiac outcomes.

More recently, Poreba *et al*. (2010), in a study of 171 men occupationally and chronically exposed to heavy metals and 19 healthy controls, found that people with pre-existing hypertension when exposed to lead had more severe and frequent cardiovascular complications, as compared to patients with hypertension not exposed to lead (Poreba *et al.* 2010b). In their cohort of lead-exposed individuals, they found positive correlations between the severity of cardiovascular complications due to hypertension and pulse pressure and blood lead levels.

A recent review by Navas-Acien *et al*. (2007) (Navas-Acien *et al.* 2007) found that in occupational prospective cohort studies by Robinson (1974) (N=1,252) and Tollestrup *et al*. (1995) (N= 1,097) and retrospective cohort studies by Malcolm (1971), Cooper *et al*.(1985), Belli *et al*. (1989), and Wilczynksa *et al*.(1998) there was no significant association of lead exposure and heart disease (Navas-Acien *et al.* 2007). They also reported that in proportional

mortality studies by Alexieva *et al*. (1981) of Bulgarian smelter workers had a RR of 5.60 (1.68–18.6) while a similar study among Australian smelter workers by McMichael *et al*. (1982) had a RR of 0.95 (0.67–1.35) when workers were compared to the general population. Other studies reported by Navas-Acien *et al*. (2007) have been reported here already. In a few studies covered by Navas-Acien *et al*. (2007), such as those by Sheffet *et al*. (1982) and Michaels *et al*.(1981), there was protective effect of lead exposure for heart disease when lead exposed workers were compared to the general population, probably as a result of healthy worker effect (Navas-Acien *et al.* 2007). In a study of 4,114 male lead workers by Gwini *et al*. (2012) (Gwini *et al.* 2012), the risk of ischemic heart disease was found to be not significant (SMR 0.95, 95%CI: 0.76, 1.19).

b. General population studies: In a study among men aged 40-59 years in the British population (n=7,371), Pocock *et al*. (1988) found an OR of 1.1 among the higher category of lead exposed (95% CI: 0.4, 1.8) to reference (<0.6 µmole/L) after adjusting for age and smoking (Pocock *et al.* 1988). Kromhout (1988), in a study of 152 men aged 57 to 76 years in the town of Zutphen, the Netherlands, did not find a significant association between blood lead level and coronary heart disease (n=26) in univariate or multivariate models, with the highest group having a RR of 1.34 (95% CI: 0.46,3.94) times that of the lowest group (Kromhout 1988). Moller and Kristensen, in a 1992 longitudinal study of 1,052 Danish survey respondents, found a non-significant association between blood lead level and coronary heart disease (HR=1.58 , p=0.15), and lead

exposure and CVD (HR=1.1, p=0.74) (Moller *et al.* 1992). Among the studies Navas-Acien *et al*. (2007) (Navas-Acien *et al.* 2007) looked at in their comprehensive literature review of lead exposure and CVD, they found little or no association in articles by Pan *et al*. (1993) (16 cases and 16 controls), Mansoor *et al*. (2000) (65 cases and 65 controls), Tsai *et al*. (2004) (68 cases and 68 controls), and Kosmala *et al*. (2004) (33 cases and 18 controls), in the general population. Gustavsson *et al*. (2001) in a population based matched case control study of 45-70 years of age in Stockholm with 1,335 cases and 1,658 controls found the adjusted relative risk of myocardial infarction was 0.88 (95% CI: 0.69, 1.12) among highly exposed and 1.03 (95% CI: 0.64, 1.65) among intermediately exposed as compared to unexposed group (Gustavsson *et al.* 2001). Dulskiene V. (2003) in a case-control study of 579 male cases (25-64 year old) of myocardial infarction, treated in Kaunas hospitals and 1,777 controls of the same age group without ischemic heart disease found an adjusted OR of 1.12 (95% CI:0.76, 1.40) in those with residential exposure to ambient lead concentrations, exceeding 0.225 $\mu g/m^3$, to those with lower lead concentrations (Dulskiene 2003).

Jain *et al*. (2007) (Jain *et al.* 2007) have studied bone lead exposure and incident heart disease in a longitudinal study of 837 men in the general population (VA Normative Aging Study) followed over a 11 year period from 1991-2001. They found 1 SD increase in blood lead level was associated with 1.27 fold increased (95% CI: 1.01, 1.59) risk for ischemic heart disease, and both bone and blood lead at baseline were significantly associated with the occurrence of heart disease over the follow-up period. Further support for a link between lead and

heart disease has also recently been demonstrated in two other studies in this same population. Park *et al*. (2006) (Park *et al.* 2006), in a study of 413 elderly men in the same population, found a significant association between bone lead and heart rate variability as well as metabolic syndrome. Perlstein *et al*. (2007) (Perlstein *et al.* 2007), in a cross-sectional study of 593 men, found a significant association between bone lead, but not blood lead, and pulse pressure ($p<0.001$) in this same population and increasing quintiles of tibia lead was associated with increased pulse pressure (p-trend=0.02). Alghasham *et al*. (2011), in a study of 55 consecutive hypertensive male patients between ages 24-59 years found statistically higher blood lead levels as compared to controls, probably mediated through ACE activity (Alghasham *et al.* 2011). Weisskopf *et al*. (2009) (Weisskopf *et al.* 2009), in a study of 868 male veterans who were part of the Normative Aging Study, found a cardiovascular mortality (n=137 deaths) adjusted hazard ratio of 5.63 (95% CI: 1.73, 18.3) comparing the lowest tertile, to the highest tertile of bone lead. They also found after adjusting for age, race and smoking, the HR for ischemic heart disease mortality (n=62 deaths) in the highest tertile was 8.37 (95% CI: 1.29, 54.4). Khalil *et al*. (2009) (Khalil *et al.* 2009), in a study of 533 women aged 65-87 years enrolled in the US Study of Osteoporotic Fractures, found that women with BLL ≥8 μg/dl had significantly higher coronary heart disease mortality, as compared to those with BLL <8 μg/dl (HR 3.08, 95% CI:1.23, 7.70).

Additional information comes from four mortality follow-up studies of the NHANES II and NHANES III populations (Jemal *et al.* 2002, Lustberg *et al.*

2002, Menke *et al.* 2006, Schober *et al.* 2006). Lustberg *et al*. (2002) (Lustberg *et al.* 2002), with 4,292 participants of NHANES II, found increased mortality from circulatory diseases (RR, 1.39; 95% CI, 1.01-1.91). Menke *et al*. (2006) (Menke *et al.* 2006), using 13,946 participants of NHANES III followed for 12 years, found participants in the highest tertile compared to the lowest, had a HR of 1.55 (1.08 to 2.24; p-trend across tertiles=0.003) for cardiovascular mortality. In addition, they found blood lead level was significantly associated with myocardial infarction. Similarly, Schober *et al*. (2006) (Schober *et al.* 2006), using the NHANES III data, followed 9,757 participants who were 40 or older at baseline, for an average of 10 years, and found overall CVD mortality relative risk of 1.20 (95% CI, 0.93–1.55) for people with blood lead levels of 5–9 μg/dl and 1.55 (95% CI, 1.16–2.07) for those with blood lead levels of ≥ 10 μg/dl (test for trend, $p <$ 0.01) after adjusting for a variety of risk factors, including SES and smoking.

In summary, in spite of explanations and evidence of possible biological pathways, there is insufficient epidemiological evidence to support the causal association of lead exposure and subsequent development of cardiovascular disease warranting further investigation of such an exposure.

5. Nonmalignant Kidney Disease

13% of US adults have diagnosed chronic kidney disease, with increasing numbers each year (Coresh *et al.* 2007). High levels of lead exposure (e.g., >40 μg/dl) can lead to an increased chronic kidney disease (U.S. Environmental Protection Agency 2005). Evidence has come from children poisoned by lead in Queensland, Australia (Henderson 1955), from moonshine alcohol drinkers (Steenland *et al.* 1990), and from

highly exposed industrial workers. Regarding the occupational cohort studies, Cooper *et al*. (1985) (Cooper *et al.* 1985), in a study of 4,519 male battery plant workers and 2,300 male lead production workers, found SMRs for chronic kidney disease (nephritis) of 2.22 (95% CI 1.55-3.45) and 2.65 (95% CI 1.14-5.22) in cohorts of battery and lead smelter workers with mean blood leads of 65 µg/dl and 50 µg/dl respectively, in the 1950s. This same study also found statistically significant increases in hypertensive disease mortality, including hypertensive nephritis, in both groups of workers. Steenland *et al*. (1992) (Steenland *et al.* 1992) (N=1,990) found an SMR of 1.55 (95% CI 0.66-1.39, 7 deaths) for kidney disease mortality, increasing to 2.79 for workers with 20+ years employment, among smelter workers who had a mean blood lead of 56 µg/dl in the 1970s. Cocco *et al*. (1997) (Cocco *et al.* 1997), in a study of 1,388 lead workers, found an SMR for genito-urinary disease (ICD 580-608.9) of 1.35 (95% CI 0.74-3.37). Analyses by length of employment found a significant trend of more genito-urinary disease mortality with longer employment (p=0.002), and a borderline trend for the subset of renal failure (p=0.09). On the other hand, Fanning (1988) (Fanning 1988), in a mortality study with 867 deaths of men who had relatively high occupational lead exposure, compared with 1,206 with low to no lead exposure, found a no excess of renal disease. Other occupational cohort studies did not present results for renal disease.

In recent years, there has been increasing evidence of lead's negative effect on renal function, as measured by increased serum creatinine, or decreased creatinine clearance, both indicators of poor glomerular filtration, at low exposure levels in general populations. Such effects may be early markers of subsequent chronic renal disease. The cross-sectional Cadmibel study in Belgium studied 2,000 people with a mean blood lead

of 11 µg/dl in men and 8 µg/dl in women, and found a significant association between higher blood lead and worse creatinine clearance (Staessen *et al.* 1992). Payton *et al*. (1994) (Payton *et al.* 1994) studied 744 participants in the Normative Aging Study in Boston in 1998-1991 who had mean blood leads of 8 µg/dl. Blood lead was significantly negatively associated with creatinine clearance. Other investigators using a sample of 459 men randomly selected from the participants of the Normative Aging Study looked at stored blood from 1979-1994 and found significant positive associations between serum creatinine and blood lead in cross-sectional analyses, and significant predictive effects for past blood lead on serum creatinine in longitudinal analyses (Kim *et al.* 1996). Finally, Wu *et al*. (2003) (Wu *et al.* 2003), in a study of 709 people, found significant associations between higher patella bone lead levels and lower creatinine clearance in the same population. In yet another large population study, Muntner *et al*. (2003) (Muntner *et al.* 2003) (N=15,211) studied the NHANES III population (mean blood lead 4 µg/dl in the late 1980s) cross-sectionally, and found a strong positive dose-response between serum creatinine and blood lead in hypertensives, but not in non-hypertensives. Hypertension can be both a cause and a consequence of chronic renal disease. Multiple studies have demonstrated the development of or worsening of kidney function among those exposed to lead even at low doses of lead exposure (Chia *et al.* 1995, Lin *et al.* 2001a, Lin *et al.* 2001b, Lin *et al.* 2003, Weaver *et al.* 2003, Tsaih *et al.* 2004, Lin *et al.* 2006, Weaver *et al.* 2009). There are a large number of studies of occupational exposure and renal function. The recent EPA Air Quality Criteria for Lead lists 39 such studies (U.S. Environmental Protection Agency 2005). Most are cross-sectional, with only a few being longitudinal. They assess renal function by creatinine clearance, serum creatinine or

BUN, all clinical indicators of impaired glomerular filtration, or by early markers of kidney disease, such as excretion of small proteins, like β-2-microglobulin or RBP (retinol binding protein), or markers of cytotoxicity such as NAD. The prognostic significance of these early markers is not known. Although not entirely consistent, the majority evaluating small protein excretion or NAD found higher levels in the lead-exposed population, compared to non-exposed controls. The same is true for evaluation of glomerular function evaluated via serum creatinine, creatinine clearance, and BUN (blood urea nitrogen), although here paradoxically there are a few studies which show lead-exposed worker with higher creatinine clearance than the non-exposed. The EPA authors note that this may reflect a phenomenon of hyper-filtration in response to renal stress, a phenomenon seen in diabetic and hypertensive patients in other settings. Longitudinal animal studies indicate that such hyper-filtration leads to later more severe renal dysfunction, but data are lacking in humans.

A recent comprehensive review of lead-related nephrotoxicity was published by Ekong *et al*. (2006) (Ekong *et al.* 2006). These authors conclude that lead contributes to nephrotoxicity, even at blood lead levels below 5 µg/dl, especially in people with other illnesses, such as hypertension and diabetes. A recent study by Weaver *et al*. (2005) (Weaver *et al.* 2005), of 803 occupationally exposed Korean workers with past exposure, found an association between bone lead and uric acid, particularly in older workers. The authors suggest that uric acid may play a role in lead nephrotoxicity. Another recent matched case-control study of ESRD of African-Americans (55 cases and 53 matched controls) (Muntner *et al.* 2007) found suggestive but not statistically significant evidence of a lead risk; median bone lead in the tibia was higher in cases than controls ($p=0.13$).

Another study, comparing highest versus lowest quartiles of environmental lead and cadmium exposure, among 14,778 NHANES participants found significant associations between these exposures and albuminuria (OR 2.34, 95% CI: 1.72, 3.18), reduced eGFR (OR 1.98, 95% CI: 1.27, 3.10), and for both outcomes (OR 4.10, 95% CI: 1.58, 10.65) (Navas-Acien *et al.* 2009). A recent longitudinal study by Weaver *et al*. (2009) (Weaver *et al.* 2009), on 537 current and former lead workers assessing lead exposure and renal function, found decreases in serum creatinine and increases in calculated creatinine clearance in men, and these changes were maximum among those with maximum decline in blood lead level ($p<0.001$). They also found significant associations of blood and bone lead with changes in renal function, indicating the nephrotoxic effects of lead exposure (Weaver *et al.* 2009). In an earlier case control study of 803 lead workers and 135 controls, Weaver *et al*. (2003) (Weaver *et al.* 2003) had found significant associations between moderate lead exposure among Korean lead workers and kidney function especially in older workers. A decade earlier, Chia *et al*. (1995) (Chia *et al.* 1995), in their case control study of 137 lead-exposed subjects and 153 controls, found that a threshold of 700 µg/L for blood lead level may not prevent the occurrence of lead nephropathy.

In conclusion, the epidemiology literature indicates that high levels of lead can cause kidney disease. Furthermore, other occupational studies and environmental lead exposure studies suggest that lead impairs kidney function at lower levels, and that this may result in later chronic kidney disease.

CHAPTER 3. RESEARCH DESIGN AND METHODS

Overall Study Design and Hypotheses of Interest

The first study is a record-based retrospective cohort mortality study, while the second is a record-based cohort study of the incidence of end-stage renal disease (ESRD), both based on pooled surveillance data from 11 states participating in the Adult Blood Lead Epidemiology and Surveillance (ABLES) program. For our third study, we compared survival patterns among patients with ESRD. The eleven states were chosen, because they were those with a large number of lead-exposed participants in their surveillance data base, and were willing to collaborate. Data were collected from the first year of testing for which computerized data was collected, through the most recent date.

The hypotheses of interest were as follows:

1) Subjects with documented exposure to lead, via a blood lead measurement, have higher rates of death from several diseases (such as lung, stomach, brain and kidney cancer, cardiovascular disease, stroke and non-malignant kidney disease) and higher rates of ESRD incidence compared to the US population (external comparison).
2) Subjects with high blood lead levels have higher rates of death from several diseases (such as lung, stomach, brain and kidney cancer, cardiovascular disease, stroke and non-malignant kidney disease) and increased rates of ESRD incidence compared to subjects with low blood lead levels (internal comparison).

3) Among subjects with a diagnosis of ESRD, those with higher blood lead levels have higher mortality than subjects with low blood lead levels, after accounting for confounders and effect modifiers.

Study Population: Adult Blood Lead Epidemiology and Surveillance (ABLES) Program

The population for this study was comprised of participants registered in eleven state registries of participants with blood lead measurements, via the Adult Blood Lead Epidemiology and Surveillance (ABLES) program (Roscoe *et al.* 2002). The ABLES program is sponsored by the US CDC's National Institute for Occupational Safety and Health (NIOSH). The ABLES program is a state-based surveillance program of laboratory-reported blood lead levels of adults with the objective to *build state capacity to initiate, expand, or improve adult blood lead surveillance programs which can accurately measure trends in adult blood lead levels and which can effectively intervene to prevent lead over-exposures*. Started in 1987, with 4 states, by 2011, the ABLES program covered 41 states.

States participating in the ABLES program are required by law for laboratories conducting blood lead tests to report their results to the state health department, whether these are occupational or non-occupational. The majority of these tests are conducted on occupationally-exposed individuals (for example in the large California data base, over 99% of the subjects work on lead-related occupations), often motivated by the fact that OSHA requires that workers exposed to high levels of lead in the air (>30 $\mu g/m^3$) undergo mandatory blood lead testing. In addition, some companies routinely test their

workers for blood lead, regardless of measured air levels. Certain states (like California) also have health care providers routinely testing for blood lead levels among women attending health care centers or ante-natal checkups (Personal communication with Susan Payne, May 2013). These blood lead levels would also be reported by the state laboratories to the ABLES program. The current definition of elevated blood lead level as per the ABLES program, as of 2009, is a blood lead concentration ≥ 10 micrograms/ deciliter (µg/dl). The public health goal of the ABLES program is to reduce the numbers of adults with blood lead levels ≥ 10 µg/dl. The ABLES program helps identify and institute interventions that help achieve this goal.

According to current OSHA regulation, workers exposed to >30 $\mu g/m^3$ of lead in the air, averaged over an 8 hour period, triggers mandatory testing and surveillance. OSHA also mandates that no employee should be exposed to lead at concentrations greater than 50 $\mu g/m^3$, averaged over an 8-hour period. Current OSHA standards require workers with BLLs ≥ 50µg/dl in the construction industry, or BLLs ≥60 µg/dl in the general industry, be removed from further exposure to lead and their blood lead levels be monitored. Workers are allowed to return to work once their BLLs are < 40 µg/dl. To provide perspective to these numbers, the current geometric mean BLL of all US adults is 1.4 µg/dl (National Institute for Occupational Safety and Health 2012). It is not known what percentage of ABLES subjects were tested due to the OSHA requirement, or simply because they were occupationally exposed at some level and either requested to be tested or their employer recommended they be tested; some BL tests were also likely for non-occupationally exposed individuals (e.g., pregnant women).

The major source of exposure for the ABLES population, however, was presumed to be occupational for the ABLES population, especially for those with blood lead levels above background (e.g. above 5 µg/dl), which represented 75% of our cohort. Industry and occupation data were collected for about 80% of those with blood leads over 25 µg/dl in the ABLES population (these represented about 50% of our cohort), but only sporadically for those with lower blood lead levels. Among those with blood leads over 25 µg/dl, NIOSH estimates that about 70% of the exposures are occupational (National Institute for Occupational Safety and Health 2012). Within the occupational exposures, the most common come from workers in the storage battery industry (36%), followed by lead smelter (10%), primary batter manufacturing (8%) and remodeling construction (paper hanging) (7%). Among non-occupational exposure above 25 µg/dl, the most common were due to exposure at shooting ranges (36%), followed by remodeling/construction (10%) (Centers for Disease Control and Prevention 2011a).

The ABLES program has adopted a surveillance case definition of an adult aged ≥16 with a venous blood lead level (BLL) of ≥ 25 µg/dl of whole blood. A few states only require reporting of cases with BLLs ≥25 µg/dl, or have required reporting of those with lower BLLs only more recently. Even when restricting to BLLs ≥ 25 µg/dl, the BLL distribution among our study population was lower than the industrial lead cohorts that have previously been studied for mortality outcomes. Historical industrial cohorts have reported mean blood lead levels in the 40-80 µg/dl range.

The variables of interest for the current study, and available from all participating states for all years, included subjects' name, sex, date of birth, year of test, and BLL. Additional variables of interest were only available from 2002 onwards from some states,

including race, industry, and source of lead (occupational or other); this information was typically only available for subjects with BLLs≥25 µg/dl.

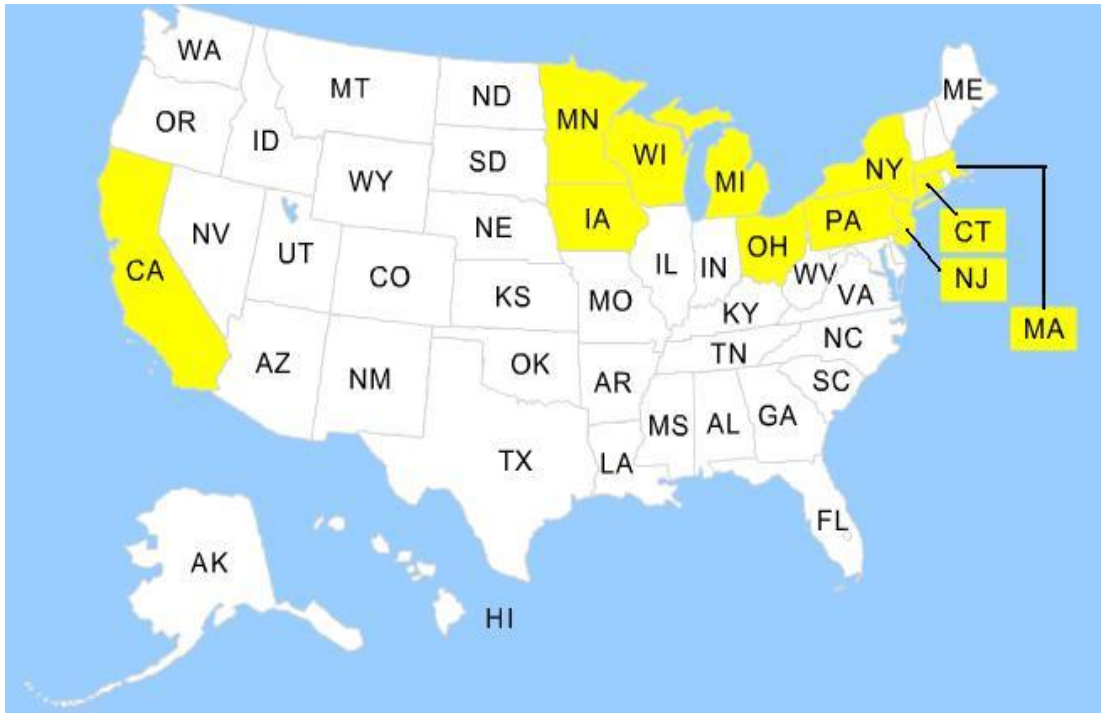
Analytic Cohort Selection

The number of states in the ABLES program has varied over time. In 2012, there were 44 states reporting blood lead levels to NIOSH up from about 25 in the period 1987-2001. For the current study, we chose all states (n=11) with relatively long periods of ABLES data with health departments that were willing to participate. The 11 states included in the present analyses were California, Connecticut, Ohio, Iowa, New Jersey, New York, Massachusetts, Wisconsin, Michigan, and Pennsylvania. Health departments from two additional states, Alabama and Washington, were unable to participate. Washington's health department had difficulties with Institutional Review Board approval and was unable to send any data. Alabama's health department agreed to participate, but despite repeated requests, our contacts did not submit data in time for inclusion in the current analyses (i.e., by end of 2011).

Table 3.1 Year of start of data collection in each state

	New York	Connecticut	Massachusetts	Wisconsin	Iowa	Michigan	Ohio	California	Pennsylvania	Minnesota	New Jersey
1st year of testing	1982	1985	1991	1988	1992	1996	1992	1987	2000	1991	1985

Figure 3.1 Map of states in our cohort

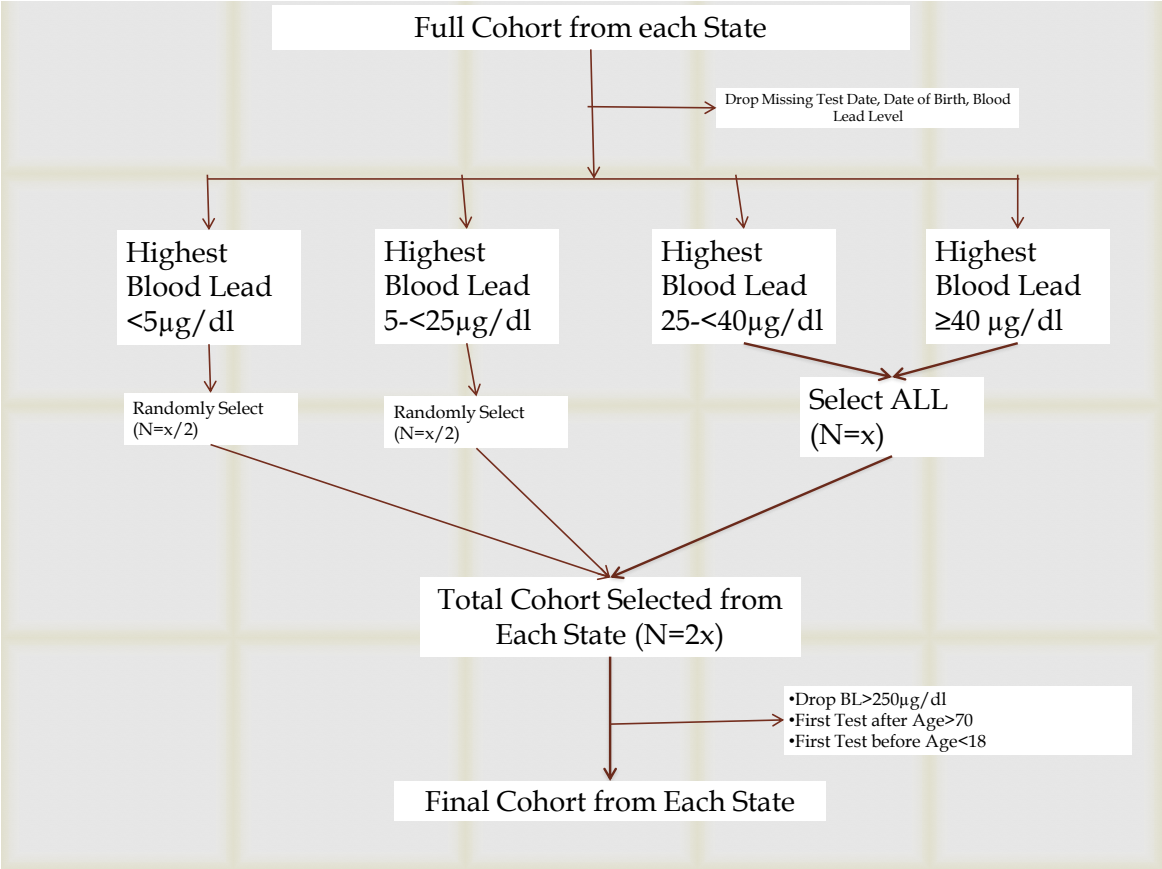


We constructed our cohort as follows. We dropped any subject missing date of birth or missing date of blood lead testing. To minimize the number of subjects with non-occupational sources of lead exposure, we excluded any subject tested for the first time after the age of 70 and before the age of 18. We also dropped subjects with tests before 1987 and after 2005. Subjects being tested after 2005 are probably still working and would not have enough time to develop the outcome. Subjects prior to 1987 were dropped to ensure uniformity of follow-up across states, as the ABLES program started in 1987 and most states did not collect data prior to 1987 in a uniform manner.

Subjects were grouped into 4 categories according to their highest ever recorded blood lead level: group 1: 0- <5µg/dl, group 2: ≥5µg/dl - <25 µg/dl, group 3: ≥25 µg/dl - <40 µg/dl and group 4: ≥40 µg/dl. Thus, each subject was assigned a lead category corresponding to the highest lead category ever attained (i.e., for those with multiple tests over time). For feasibility of matching ABLES subjects with the National Death Index (NDI) and United States Renal Data System (USRDS) for end-stage renal disease (ESRD) incidence, we aimed to reduce the total number of subjects in our cohort while not impacting the power of our study. Most states had a larger number of people in categories 1 and 2 than in categories 3 and 4. Given the high cost of obtaining matched data from the NDI and USRDS, we had to reduce the numbers selected from lead categories 1 and 2. To do this, we took the total number of unique subjects in lead categories 3 and 4 by state and randomly sampled an equal number of subjects from lead categories 1 and 2 (50:50 sample from lead categories 1 and 2) from that state. These subject records were then sent to NDI for mortality follow-up and USRDS for ESRD incidence follow-up (discussed in more detail in the Outcome Data section below).

We also excluded subjects with blood lead levels greater than 250 µg/dl, as these values were considered implausible. We then further searched for duplicate records across states using combinations of SSN, last name and date of birth and found 2300 duplicate records (i.e., for subjects with tests in different states). These records were assigned the same subject identity number across states for uniformity. The duplicate records that we were unable to distinguish were dropped from the analysis.

Figure 3.2 Selection of the cohort



Exposure Data

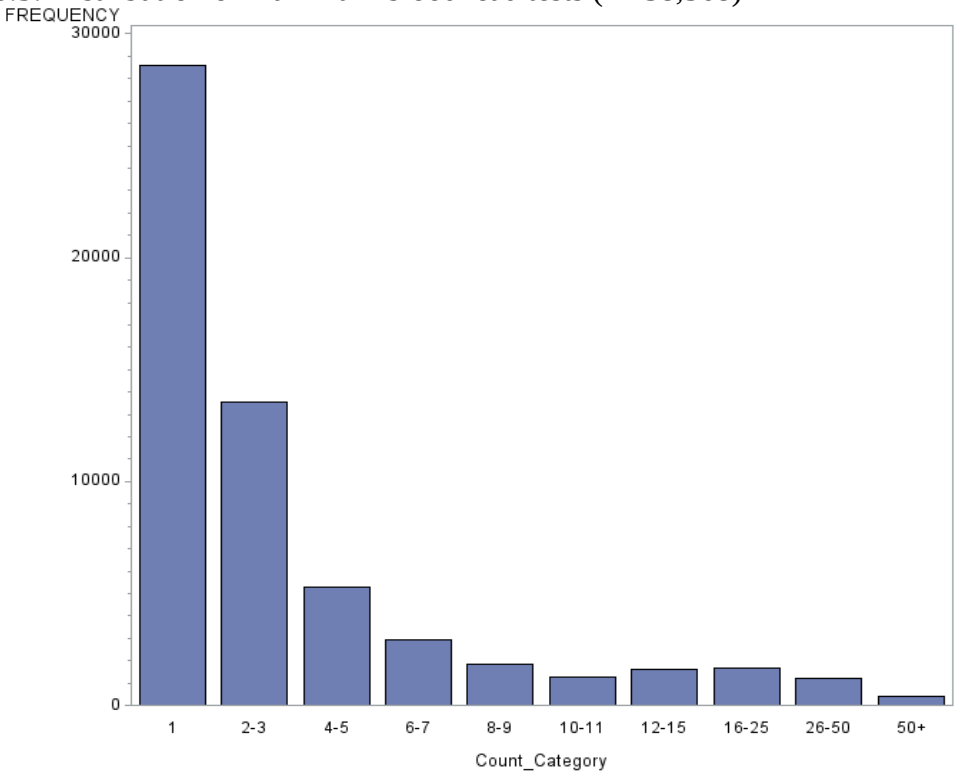
Exposure data for this study consisted of the BLL for each participant, starting with the first year that participant entered the ABLES registry. The major route of

exposure was presumed to be inhalation in an occupational setting, as discussed above. A limitation of these exposure data was that each BLL record only represents exposures for one point in time (at the time of the test). For the subset of subjects (50%) with multiple tests, we found that blood lead levels tracked well over time. For example, 18% of subjects had more than one BL test over time, for which BLLs did not change category. Another 24% changed category over blood tests, but by only one category. Only 7% of the cohort changed blood lead category by more than one BL level. Thus, for those subjects (50%) with only a single blood lead level measurement, we are reasonably confident that their single measurement is representative of their longer term BLLs. It is important to acknowledge, however, that a complete work history was not available for these subjects. Blood lead levels for those exposed occupationally represent recent exposure; the half-life of lead in the blood under steady state conditions has been estimated as 3 weeks (Fischbein 1998). Despite this limitation, the strength of these data are the solid documentation of an internal exposure (dose) level often considerably above background for the US population, which currently has a geometric mean of <3 µg/dl for adults.

Table 3.2 Distribution of subjects changing categories (N=58,368)

Tests	Change of Category	N	Percent	Mean	Median	Maximum
Single Test	NA	28,589	48.98%	1	1	1
>1 test			0.00%			
Difference of Highest and Lowest Lead Categories	0	10,577	18.12%	3.92	2	98
	1	14,178	24.29%	8.33	5	169
	2	4,594	7.87%	13.86	8	243
	3	430	0.74%	14.15	8	198
OVERALL		58,368	100.00%			

Figure 3.3: Distribution of maximum blood lead tests (N=58,368)



1. Selection Bias: We do not think that the selection of our cohort is related to the diseases of interest. ABLES participants generally have their blood tested based on recommendations of their employers, due to being exposed to lead. This is unlikely to be related to any chronic disease among the subjects.

Selection biases could also occur if the workers selected for this study from the eleven states were not representative of all lead-exposed workers with respect to their exposure-response relationship, as might occur if they differed for an unmeasured confounder. One such unmeasured confounder might be geographical region, i.e., those living in the states selected for the study might differ from other ABLES workers in other states not selected for some confounder which varied by state. Using the data from the year 2001(Robert J. Roscoe 2002, Roscoe *et al.* 2002) for 24 states reporting to ABLES that year, the 11 states proposed for this study represented 77% of the workers with BLLs ≥25 μg/dl reported in all 24 states. Male death rates per 100,000 age 15+ in the eleven states in question 1990-1998 are similar to the 13 other states, and to the US (age standardized). Death rates in the 11 included states and the 13 non-included states are reasonably similar, and both are similar to the US.

2. Misclassification of Exposure: We classified exposure into 4 groups based on highest category of lead exposure ever achieved. A limitation of this exposure data is that it existed for only one point in time (at the time of the test) for 50% of the subjects, and the rest had multiple tests across several years. Thus, it is possible that there may be misclassification of exposure for those with a single blood lead level. However, among those with multiple tests, we found that multiple blood lead level tracks well with little

change over time. About half of the cohort (49%) had only one blood lead test, while another 18% had more than one, but did not change category. Another 24% changed category over blood tests, but by only one category. Only 7% of the cohort changed blood lead category by more than one BL level. Thus, for those with single blood lead level measurement, we are reasonably confident that their single measurement is similar to other measurements of BLL, had they been taken.

3. Blood Lead vs. Bone Lead:

Bone lead, a measure of the lead content in bones, can be measured from different bones. Most commonly we use tibia or patella to measure bone lead. Since bone lead levels are dependent on the activity levels and bone remodeling/resorption (during healing of fractures, growth spurts, pregnancy, post-menopause (Webber *et al.* 1995, Korrick *et al.* 2002), hyperthyroidism (Goldman *et al.* 1994) etc.) that takes place, bone lead levels may vary with the bone being used to measure lead levels. However, blood lead levels are a measure of circulating lead that indicates acute changes in external and internal (mobilization from tissues) lead exposure, while bone lead reflects long-term exposure. Thus, for chronic disease development, bone lead would presumably be most relevant. Measured together, these would form a valuable estimate of total lead exposure in the body. However, bone lead is both expensive and difficult to measure, and many studies measure only blood lead. Multiple blood lead measures integrated over time form an acceptable alternative for cumulative lead exposure and bone lead measurement are not needed, as they are found to be well-correlated with bone lead(Somervaille *et al.* 1988, Roels *et al.* 1995). In the Nurses' Health Study (NHS), Korrick *et al*. (2002) (Korrick *et al.* 2002), using a sample of 264 Bostonian women, found that blood lead

levels track very well independently with both patella and tibial lead levels. Thus, the use of blood lead levels can be justified in studies associating lead levels to chronic diseases. However, another study of approximately 1,000 subjects, 50–70 years of age, had a mean blood lead level of 4 μg/dl and mean tibia lead level of 19 μg/g, with a Pearson's r correlation of only 0.12(Schafer *et al.* 2005, Martin *et al.* 2006). Despite these conflicting data on the correlation of bone and blood lead, BLL is commonly used instead of bone lead in epidemiologic studies, because bone lead measurements are invasive, expensive, and time consuming, and generally not feasible for large cohort studies.

Blood lead levels are usually measured in whole blood collected by venipuncture (Schlenker *et al.* 1994), and laboratories use different techniques to measure BLLs. Commonly, graphite furnace atomic absorption spectroscopy is used, which has a limit of detection (LOD) of 1 μg/dl. Other techniques are anodic stripping voltammetry, which has an LOD of 5 μg/dl. These techniques are well-standardized with well-established quality control measures. Multiple studies have indicated the half-life of lead in blood is 35 days. This is in part a reflection of the average life span of RBC at any point in the blood. Their findings are based on short-term exposure to lead. When lead exposure is chronic or long-term, when exposure stops, there is an initial rapid drop in blood lead levels reflecting the partial clearance from soft tissues and blood. This is followed by a slower rate of clearance reflecting clearance from blood with partial replacement of blood lead from other organs and tissues, such as bone. Hence, BLLs reflect an acute short-term external exposure, release of lead from internal stores, such as bone, but usually a combination of the two.

Bone lead is usually measured from tibia or patella. Autopsy studies have shown that skeleton contains 90-95% of lead stores in adults and 80-95% in children (Schroeder *et al.* 1968, Barry *et al.* 1970, Barry 1981, Hu *et al.* 1989). Rabinowitz *et al.* (1976) (Rabinowitz *et al.* 1976) showed that approximately 15% of circulating lead per day is stored in bones, replacing calcium during the normal bone remodeling process. Cortical bone has a half-life of decades, while trabecular bone has a half-life of years to decades. Thus, cortical bone lead levels are a good reflection of long-term lead exposure. Bone lead can be measured on autopsy, biopsy or by non-invasive measures, such as *in vivo* K-shell X-ray fluorescence (KXRF) (Chettle *et al.* 1991, Todd *et al.* 1992a, Todd *et al.* 1992b, Landrigan *et al.* 1994, Todd *et al.* 1994, Hu *et al.* 1995, Hu *et al.* 1998). Trabecular bone, with a shorter and more variable half-life, is a bit unreliable as a measure of cumulative lead dose. But trabecular bone may reflect a more bioavailable store than cortical bone lead (Hu 1998). However, bone lead measurement by KXRF techniques require exposure to low dose gamma radiation (Hu *et al.* 1998).

Lead can also be measured from serum or plasma, as this represents the portion of body lead that is more readily available to produce toxic effects (Cake *et al.* 1996). However, plasma or serum lead levels constitute a very small component of body lead (<1% of whole blood level). Measurement of lead from plasma or serum is difficult, as it requires special methods for collection, processing, quality control and measurement. These samples are also prone to contamination, due to hemolysis of RBCs which contain >99% of lead in blood (Smith *et al.* 2002). Due to the transient nature of these lead levels, it is difficult to associate them with chronic health effects.

Outcome Data

We obtained outcome information from two sources: the National Death Index and the United States Renal Data System.

1. National Death Index: We used name, date of birth, gender, race (when available) and SSN (when available) for matching with the NDI database until the end of 2010, to obtain data on date and cause of death (underlying and multiple). For the three states, that independently sent their data NDI, follow-up ended in 2009. To determine if a match with the NDI was a true match from amongst the multiple matches reported by NDI, we only selected those who were assigned a status code of 1 by NDI, indicating a high probability of a match. If person's last blood lead date was after their date of death, then the match was false, and we dropped all information received from NDI i.e. these subjects were considered as alive. If there were multiple matches with status code 1, we selected the one the NDI reported as an exact match. If there was no exact match, we sorted all the status codes=1 by probability score. If highest probability score was ≥ 40 and state of death was the same as the state where that subject was tested, then we selected that observation. If there were multiple matches meeting this criterion, then we selected the one with the higher probability score of match. If we are unable to select a match based on the above criteria, we dropped those observations entirely from the final dataset, to avoid misclassification of outcome.

2. United States Renal Data System: USRDS was reasonably certain regarding their matches. ESRD occurs when the kidneys fail. Thus, ESRD patients can be thought of as a subset of those with acute or chronic kidney disease. Kidney failure requires either dialysis or transplant as a treatment. Data on ESRD are collected annually by the United

State Renal Data System (USRDS). The USRDS is funded directly by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in conjunction with the Centers for Medicare & Medicaid Services (CMS, formerly HCFA). The USRDS publishes the number of annual cases, and both incidence and prevalence data. Medicare began paying for dialysis and transplant in the US in 1972, via the Health Care Financing Administration (HCFA). The great majority of cases of end-stage renal failure take advantage of this because treatment is quite expensive. Validation studies have shown that about 95% of all US patients with ESRD are in the USRDS system (1992, USRDS 1992). Incidence rates of ESRD are available since 1973 and currently go through 2003. We expect to use such rates through 2005. The rates are age, race, sex, and year specific. USRDS used name, SSN, race, gender and date of birth, analogous to the matching done by NDI. Similar matching of other occupational cohorts has been done in the past (Calvert *et al.* 1997, Steenland *et al.* 2001, Radican *et al.* 2006). Our initial matching with the USRDS gave us follow-up information until 31st December, 2008. Due to confidentiality issues, some states (~15% of the cohort), namely Wisconsin, Massachusetts and Michigan, sent their data independently to the NDI and USRDS. We received de-identified data for these observations directly from the states, preventing further follow-up with USRDS. In 2012, we sent our dataset to USRDS again for follow-up information until 31st December, 2010.

In addition to the standard list of matching variables, for the third paper, we also requested the USRDS to provide detailed information on the following Core Standard Analysis Files (SAF) data:

a. Treatment History (RXHIST file)
b. Medical Evidence (MEDEVID95)
c. Medical Evidence (MEDEVID05)
d. DEATH
e. Transplant (TX)
f. Patients

The files provided data on the following variables of interest: age at time of diagnosis, Hispanic ethnicity, race, co-morbidities, type of insurance, glomerular filtration rate (eGFR) at diagnosis, type of ESRD, transplantation status and details among others. The USRDS also sent us additional information on vital status for people with ESRD, which we used for paper three only.

Analytic Plan

For our first study, we restricted the dataset to male participants. because females represented only 7% of the deaths and were concentrated more heavily in the lowest blood lead category, which is likely to have included a higher percentage of non-occupational blood leads, especially among women tested for lead during pregnancy (personal communication, Susan Payne, California ABLES, May 2013). The NIOSH Life Table Analysis System (NIOSH, LTAS, Version 3.0) was used to calculate person-years of exposure and rates of death for the cohort, and then compare these rates with those of the US population via standardized mortality rations (SMRs), adjusted for age, race, sex, and calendar time (Robinson *et al.* 2006, Schubauer-Berigan *et al.* 2011). SMRs are calculated for 92 specific causes of death. National rates were used, rather than 11 different state rates, for convenience, and because mortality rates in these 11 states (including the large states of Pennsylvania, Ohio, New York, New Jersey, and

California), as a whole, tend to reflect overall US rates. Life table analyses used the NIOSH life table system (LTAS) for personal computers 311, which in its updated version has 119 separate death categories for multiple causes (including all the categories of *a priori* interest), and also has state comparison rates for these death categories. The NIOSH program covered the transition from the ninth to the tenth ICD revision (which occurred in 1999). The NIOSH program used the ICD code at time of death and allocated deaths in the appropriate death categories across revisions 265. NIOSH LTAS required gender and race (white vs. non-white) as stratification variables in the analysis. Those subjects missing gender (0.2%, n=116) were classified as male. Large numbers of subjects (69%) were missing race and were classified as white. Among those with known race, 80% were white. Hence approximately 12% of non-whites in our study were likely to have been misclassified as white, potentially causing some bias in our results.

We calculated SMRs for all blood lead categories (1 through 4) and all categories combined, as well as stratified by time since first exposure (0-5, 5-10, 10+ years). Person-time at risk began at time of first blood test for everyone. All person-years were assigned to the highest blood level; most people did not change blood lead category. We considered an analysis using a time-dependent categorization of blood lead level, where subjects could change categories across time, but as we did not have complete blood lead histories, we felt such an analysis was not justified. Another reason against such an analysis was the relatively few numbers that changed categories over time (7% changed 2 or more categories over time). This also follows from the metabolic pathway of lead in the body where duodenum is both the site of absorption and excretion. Hence, people would tend to lose very little lead over time, and thus remain in the same category.

In internal analyses, we calculated Standardized Rate Ratios (SRRs) using Poisson models (log person-years offset, with scaled standard errors to adjust for over-dispersion), adjusted for gender, race, age category, and calendar period, and comparing each lead category (5 to <25 µg/dl, 25 to <40 µg/dl, and 40+ µg/dl) to the reference category (<5 µg/dl) with SAS version 9.3 (SAS Institute, Cary, NC). Person-time and events in these analyses were grouped in 5 year age and calendar time periods, although age categories below age 50 were generally collapsed, as few deaths from chronic disease occur prior to age 50, making models unstable. In some instances, when the cause of death was rare, further collapsing of 5 year age and calendar time periods was also required. We also conducted a trend test in Poisson regression by assigning median values of highest blood lead level for each category as the category median.

We also ran several sensitivity analyses. To possibly reduce misclassification of exposure status among people who changed BLL categories, we conducted the same analysis on a subset of the people (67%) who did not change BLL categories. To determine if absence of SSN (which might have affect NDI matching resulting in misclassification of outcome) would affect the results, we conducted an analysis on the subset of the people with SSN (26%). We also further divided our blood lead category 4 (40+µg/dl), based on its median, into very high and extremely high categories (cut-point was 50µg/dl), and thus obtained 5 categories of lead exposure, namely, <5 µg/dl, 5 to <25 µg/dl, 25 to <40 µg/dl, 40- <50 µg/dl and 50+ µg/dl.

For our second study, we further split category 4 into two based on the median highest blood lead level (51 µg/dl), and thus had 5 categories of lead exposure, namely, <5 µg/dl, 5-<25 µg/dl, 25-<40 µg/dl, 40-<51 µg/dl and ≥51 µg/dl. Non-whites are known

to have much higher rates of ESRD incidence than whites. For example, age and gender-adjusted USRDS ESRD incidence rates (per 100,000) for African-Americans and whites in 2010 in the US were 92.4 and 27.5, respectively (http://www.usrds.org/reference.aspx, accessed June 9, 2013). Furthermore, in those with known race based on state data (31% of the cohort), race (white/non-white) was a confounder, in that it was strongly related to ESRD incidence, and also weakly related to BL category (higher BL categories had higher percent non-whites, 15% in the lowest BL category, 18% in the highest). Hence, the fact that 69% of our cohort was missing data on race created potential for bias. Furthermore, because race was a required field to run the NIOSH life table, we were unable to use LTAS without assigning some value for race (white/non-white) to those missing race. It should be noted, however, that we had race data on all ESRD cases from the USRDS. We chose two strategies to confront the problem of missing race.

First, we conducted an analysis restricted to the 31% (18,057) with known information on race (including the 108 ESRD cases arising from this sub-cohort). Second, we imputed race for those missing race in the entire cohort (although not for the 302 ESRD cases arising in the total cohort, for whom we had data on race), and ran a life table for the entire cohort. To do this, we first built an imputation model using logistic regression (in the SAS procedure MI) to predict race (white/non-white) among those with known race. Predictors in this model included year of birth, gender, BL category, state where tested, vital status, year of first lead test, and the presence or absence of ESRD. This model resulted in correct prediction of race for 69% of the observations, incorrect for 30%, and 'tied' data for 2% of observations. The area under the ROC curve was 0.69, indicating only moderate success in predicting race by the model, but better than

randomly assigning race to those missing it. We then used the regression coefficients from this model (again via SAS PROC MI) to generate five data sets for the entire cohort in which race was imputed for the 69% missing race. Imputation of race for those missing race was done via five Monte Carlo runs in which the coefficients from the imputation model were assumed to be multivariate normal, and a draw was made from this multivariate distribution for each person, generating a predicted probability of race (between 0 and 1) for each subject missing race, and then race was assigned for each individual using a binomial distribution based on each given probability 'p'. This resulted in five imputation data sets. The percentage non-white race in these five data sets (males only) varied only slightly, from 18% to 20%, and was similar to those with known race (17% non-white). We then ran life table analyses separately for each of these imputed data sets, and averaged the results (specifically averaged the expected ESRD cases, as the observed did not change). It should be noted that we had the exact race for all ESRD cases from USRDS, and therefore did not need to impute race for the cases. The variance of the ensuring summary race ratio assumed that the observed number of cases was Poisson distributed, and that the expected number of cases was invariate. This was not technically true, as the expected number of ESRD cases varied across the five life table runs. However, this variance was very small, and its addition to the variance of the observed did not change the resulting confidence intervals. Further, these analyses were restricted to males, because females represented only 9% of ESRD cases and were concentrated more heavily in the lowest blood lead category, which is likely to have included a higher percentage of non-occupational blood leads, especially among women tested for lead during pregnancy (personal communication, Susan Payne, California

ABLES, May 2013). Anyone with an ESRD diagnosis prior to their first test date was also dropped from the analysis.

For the third study, we created an additional category by splitting the 40+ category into two groups- 40-<50 µg/dl and 50+ µg/dl based on the OSHA cut-off for removing people from working in lead industry at 50+ µg/dl and not letting them return to such work until their blood lead level fell to 40 µg/dl. This cut-off was also based in part on the patterns seen in a prior work with the same data on the association of lead exposure and incident ESRD. Anyone who matched with the USRDS was considered eligible to be a part of the analytic cohort. However, anyone who had an ESRD diagnosis on file before they were ever tested was dropped from the analysis. Further, we also obtained data on date of death and cause of death from the USRDS, as the USRDS follows all ESRD cases longitudinally. If an observation was not declared dead by NDI, but had been so by the USRDS, we considered the person to be dead. The USRDS uses the Social Security Administration (SSA) to determine deaths. We used Cox Proportional Hazards models to evaluate association of survival pattern and lead exposure level in the five aforementioned categories among ESRD cases after adjusting for covariates, including age at first test, race, ever transplanted, glomerular filtration rate (GFR) before start of dialysis, body mass index (BMI), year of ESRD diagnosis (for cohort effect) and co-morbidities -specifically chronic obstructive pulmonary disease (COPD) and any cardiac disease. GFR and age at ESRD diagnosis were modeled as continuous variables, as they showed a monotonic trend when examined in quartiles and quintiles, respectively. BMI was divided into 4 categories – underweight (<18.5 kg/m^2), normal (18.5-<24.9 kg/m^2), overweight (24.9-<30 kg/m^2) and obese (≥30kg/m^2). *A priori* we had postulated

five variables to be predictors of outcome- lead exposure in categories, age at ESRD diagnosis, GFR, BMI and transplantation status. In addition, from the literature we identified other variables that we adjusted for in our analyses. First, we looked interaction terms of lead exposure by category and the following variables- transplantation status, age at ESRD diagnosis, GFR and race. None of the interaction terms were significant at alpha=0.05, and hence were dropped. The model without interaction terms also had a lower AIC than ones with interaction terms in them. Next, we assessed confounding. We used backwards elimination to reduce our full model to final models using Akaike Information Criteria (AIC) and p-values (those with p-values >0.1 were dropped). All variables considered *a priori* to be predictors (lead exposure by category) were retained even if they were found to be insignificant. We further assessed the proportional hazards assumption for lead exposure via interactions of lead exposure in categories with time for our final model.

Comparison of Poisson and Cox Proportional Hazards Models

1. Poisson Distribution: Named after the French mathematician, Siméon Denis Poisson (1781–1840), the Poisson distribution, is a parametric discrete probability distribution that models the probability of a given number of events occurring in a fixed interval of time and/or space, if these events occur with a known average rate and independent of the time since the last event.

Mathematically, a discrete random variable X is said to have a Poisson distribution with parameter $\lambda > 0$, if for k = 0, 1, 2, ...∞, the probability mass function (pmf) of X is given by:

$$f(k;\lambda) = \Pr(X = k) = \frac{\lambda^k e^{-\lambda}}{k!},$$ where

- e is the base of the natural logarithm (e = 2.71828...)
- k! is the factorial of k.
- $\lambda = \lambda T$, when the number of events occurring will be observed in the time interval $T = 1$.

The Poisson has a very useful property, namely, λ is equal to the expected value of X and also to its variance, hence λ has to be greater than 0.

$$\lambda = \mathrm{E}(X) = \mathrm{Var}(X).$$

Since the Poisson model is a parametric model, we can use the principles of Maximum Likelihood Estimation (MLE).

In our studies 1 and 2, if we consider the distribution to be Poisson, the λ to be the observed number of cases, then using the above property, we know the variance. This made calculation of the SMR and SRR for internal and external comparisons relatively easy. Another advantage of assuming the distribution to be Poisson was that LTAS automatically calculated the person-years of follow-up for each calendar period. This made further programming in SAS much simpler. This was especially so, as constructing

these periods by hand or in SAS would have been quite tedious, given the larger sample sizes in these papers (N=58,000+).

Since we were interested in comparing rates of mortality and incident ESRD in papers 1 and 2, respectively, we divided time into 5 year periods and calculated rates in each time chunk and compared this to rates of disease in that time period. This is especially important for diseases like lung cancer, whose mortality rates in the general US population have been changing over time. Further, these rates are available from national data and other sources in larger time chunks (5 year periods). If these were available for smaller time periods, i.e. as $\Delta t \rightarrow 0$, results using the Poisson distribution would approach those from the Cox models (see below).

2. Cox Proportional Hazards Model: Proportional hazards models, a type of model used in survival analysis, relate time to an event, to one or more exposure/predictor variables of interest that may be associated to the duration before the event. These models have two distinct parts, an underlying/baseline hazard function, denoted by $\lambda_0(t)$, and the part with the exposure/predictor variables of interest. The baseline hazard represents the changes over time (t) at baseline levels of covariates. Sir David Cox observed that if the proportional hazards assumption was satisfied (or assumed), then it was possible to estimate the parameter estimates without stating the hazard function. These models are hence called *Cox proportional hazards (PH) model or Cox models or proportional hazard models.*

Thus, when we compare two levels of a predictor, the baseline portion drops out, and we do not have to state or make assumptions regarding the distribution of the baseline hazard function. Hence, these models are semi-parametric in nature (as one part of the model, namely the baseline hazard function, is not clearly defined). Since the entire model is not completely specified, MLE approach cannot be used and in its stead, a pseudo-likelihood or partial likelihood can be constructed as below. The partial likelihood can be maximized using the Newton-Raphson algorithm. The inverse of the Hessian matrix, evaluated at the estimate of β, can be used as an approximate variance-covariance matrix for the estimate, and used to produce approximate standard errors for the regression coefficients.The Cox partial likelihood (see below) is obtained by using the Breslow estimate of the baseline hazard function.

Let Y_i denote the observed time (either censoring time or event time) for subject *i*, and let C_i be the indicator that the time corresponds to an event (i.e. if $C_i = 1$ the event occurred and if $C_i = 0$ the time is a censoring time). Then, the hazard function for the Cox proportional hazard model has the form

$$\lambda(t|X) = \lambda_0(t)\exp(\beta_1 X_1 + \cdots + \beta_p X_p) = \lambda_0(t)\exp(\beta' X).$$

This expression gives the hazard at time *t* for an individual with a particular set of explanatory variables, *X (in vector notation-covariate vector)*. Based on this hazard function, a partial likelihood can be constructed from the datasets as

$$L(\beta) = \prod_{i:C_i=1} \frac{\theta_i}{\sum_{j:Y_j \geq Y_i} \theta_j},$$ where

$\theta_j = \exp(\beta' X_j)$ and $X_1 \ldots X_n$ are the covariate vectors for the n independently sampled individuals in the dataset. This can be log transformed and maximized over β to produce maximum partial likelihood estimates of the model parameters. In the above partial likelihood, the baseline hazard has cancelled out, while the second part is free of regression coefficients and depends directly on the data through the censoring pattern. Thus, the effect of predictors estimated by any PH model may be reported as hazard ratios. The Cox model can be generalized to time varying covariates i.e. the proportional effect of a predictor may vary over time and the hypothesis of no change with time of the coefficient may then be tested.

In our third study we explored the survival patterns among ESRD cases, and the association with lead exposure in categories. Thus, the Cox PH models were ideally suited to evaluate our hypothesis.

3. Comparing Poisson to Cox PH Models: Poisson regression provides rate ratios for grouped (categorical) data, while Cox regression also estimates a rate ratio based on individual data. They will give similar results, especially when the categorization becomes finer in Poisson regression.

In Poisson models, the time chunks used for derivation of rates are usually large. If these were reduced, i.e. $\Delta t \rightarrow 0$, then Poisson model approaches the Cox PH models, if the hazards become proportional in these infinitesimally small time periods. On the other hand, if we assume constant hazard rates over fixed time intervals (Δt) or piece-wise exponential model, we can fit flexible survival models in the form of a Poisson GLMs. If

we add interaction terms between the piecewise constant baseline hazard and covariates, we can estimate time-varying effects and would not need the proportionality assumption.

To illustrate the link between these two regression models, let us suppose the baseline hazard is constant over time: $h_0(t)=\lambda$. Then the survival function can be written as

$S(t)=\exp(-\int t_0 \lambda du)=\exp(-\lambda t)$ and the density function is $f(t)=h(t)S(t)=\lambda\exp(-\lambda t)$

This is the pdf of an exponential random variable with expectation $\lambda-1$.

Such a configuration yields the following parametric Cox model $h_i(t)=\lambda\exp(x'_i\beta)$

In the parametric setting, the parameters are estimated using the classical maximal likelihood method. The log-likelihood is given by

$l=\sum i\{d_i\log(h_i(t_i))- t_i h_i(t)\}$, where d_i is the event indicator.

Up to an additive constant, this is nothing but the same expression as the log-likelihood of the d_i's seen as realizations of a Poisson variable with mean $\mu_i=t_i h_i(t)$. Thus, we can obtain estimates using the following Poisson model:

$\log(\mu_i)=\log(t_i)+\beta_0+x'_i\beta$, where $\beta_0=\log(\lambda)$.

The Cox PH model offers a lot more flexibility in terms of modeling, as it is semi-parametric in nature and does not impose a specific distribution on the outcome variable in the dataset. In addition, in Cox models a large number of predictor variables,

confounders and effect modifiers can be modeled. Thus, in our third study, we preferred to use a Cox PH model over a Poisson model.

With a limited number of covariates available in papers 1 and 2 (age, race, sex, calendar time, BL category), and with data already grouped into by these same covariates by the NIOSH life table, we chose Poisson regression for internal analyses in papers 1 and 2. In contract, for paper 3, we had more covariates for individuals, and needed more extensive modeling, than in papers 1 and 2. Hence we chose Cox regression for paper 3.

CHAPTER 4. Lead exposure and mortality among participants in a lead surveillance program.

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Abbreviations: ABLES: Adult Blood Lead Epidemiology & Surveillance

ABSTRACT

Background: Current evidence indicates that lead exposure increases blood pressure in adults, and may increase cancer risk (primarily lung and stomach).

Methods: We studied men who were part of a NIOSH-sponsored occupational lead surveillance program in 11 states, via external and internal comparisons. Blood lead (BL) categories were 0-<5 µg/dl, 5-<25 µg/dl, 25-<40 µg/dl and 40+ µg/dl, defined by highest blood lead test.

Results: There were 58,368 males with a median 12 years of follow-up (increasing from 6 years in the lowest BL category to 17 in the highest), and 3,337 deaths in the cohort. Sixty-percent of workers had only one BL test, while the remainder had a median of four tests. There was a strong healthy worker effect (all causes SMR=0.69, 95% CI 0.66, 0.71), which decreased with increasing follow-up. The 40+ µg/dl category had elevated lung and larynx cancer SMRs (1.20, 95% CI 1.03-1.39, n=174, and 2.11, 95% CI 1.05, 3.77, n=11, respectively), but did not show an excess of brain cancer (SMR 0.83, n=11), kidney cancer (SMR 0.72, n=9), stomach cancer (SMR 0.92, n=10), stroke (0.79, n=47), or heart disease (SMR 0.72, n=223). There were significant positive trends by BL categories for all causes (p=0.0001), lung cancer (p=<0.0001), COPD (p=0.02), chronic kidney disease (p=0.02), and ischemic heart disease (p<0.0001). Lung cancer SRRs by increasing BL category were 1.0, 1.34, 1.88, and 2.79. Among smoking-related causes, only lung and larynx cancer showed significant excesses in the highest lead category.

Conclusion: We found evidence of increased risk of lung and larynx cancer with higher lead exposure, with significant positive trends by increasing lead category. Positive

trends were also seen for heart disease and kidney disease death. Data are limited by lack of work history and smoking data, different follow-up time for different lead categories, and small number of deaths for some causes.

INTRODUCTION

With the EPA's establishment of permissible level of lead in the air (U.S. Environmental Protection Agency 1977), and reduction of lead use in commercially available products (particularly leaded gasoline), ambient lead exposure has been reduced. However, occupational exposure continues to be important, with the National Institute of Occupational Safety and Health (NIOSH) estimating more than 3 million workers in the US potentially exposed to lead (Rempel 1989, Staudinger *et al.* 1998). The current OSHA standard calls for removal of workers from exposure when blood lead levels are greater than 50 µg/dl, until they have levels below 40 µg/dl, although a number of authors have called for removal of workers from exposure when blood levels reach 20 µg/dl (Hu *et al.* 2007, Kosnett *et al.* 2007, Schwartz *et al.* 2007, Spivey 2007). These authors point out that current lead standards were set in order to avoid acute symptoms of lead poisoning, but do not appear protective against chronic disease outcomes, as evidenced by studies in the last 10-15 years.

Lead is neurotoxic in children (Bryce-Smith 1972, Blackwood 1975, de la Burde et al. 1975, Valdes Bolanos 1975), and can cause acute poisoning in adults (White 1975, Agency for Toxic Substances and Disease Registry 2007, Garcia-Leston et al. 2010). However, it is less recognized that lead may to cause chronic health effects in adults. Adult chronic exposure to lead has been associated with multiple outcomes, including hypertension, cancers, heart disease, non-malignant kidney disease, among others, but the evidence is not conclusive for any of them. In 2004, both the International Agency for Research on Cancer(IARC) (International Agency for Research on Cancer 2006) and

National Toxicology Program (National Toxicology Program 2004) concluded that lead was a probable human carcinogen, based primarily on lung and stomach cancer, with some suggestion of an effect for kidney and brain cancer (Baker *et al.* 1980, Fu *et al.* 1995, Kurt 1995, Lundstrom *et al.* 1997, Cocco *et al.* 1998, van Wijngaarden *et al.* 2006, Rousseau *et al.* 2007, Wu *et al.* 2012). More recently, IARC has called for further research to study the role of lead exposure in mortality in relation to cancer, to help resolve ambiguities in existing data (Ward *et al.* 2010).

Lead exposure has been associated with modest increases in blood pressure (Nawrot et al. 2002). Increased blood pressure is a risk factor for stroke and heart disease, but information on these outcomes is limited in the current literature (Navas-Acien *et al.* 2007).

Very high levels of lead in the body are known to result in kidney failure (U.S. Environmental Protection Agency 2005), but effects at low levels are less clear. A recent comprehensive review of lead-related nephrotoxicity (Ekong *et al.* 2006) concluded that lead contributes to nephrotoxicity, even at blood lead levels below 5 µg/dl, especially in people with other illnesses, such as hypertension and diabetes. However, it is not known whether such nephrotoxicity is severe enough to lead to chronic renal disease.

The objective of the current study was to evaluate the association of lead exposure and subsequent mortality, due to cancer, non-malignant kidney disease, stroke, and heart disease, using data from 11 states participating in the Adult Blood Lead Surveillance (ABLES) program, sponsored by the National Institute for Occupational Safety and Health (NIOSH).

METHODS

Data Sources/Study Participants

The Adult Blood Lead Surveillance (ABLES) program, sponsored by the National Institute for Occupational Safety and Health (NIOSH), started collecting state-level data on blood lead levels in 1987 (Roscoe *et al.* 2002). In participating states, state agencies collected data on all subjects tested in any laboratory in the state doing blood lead tests. Initially, some states gathered data only on those whose blood lead levels exceeded 25µg/dl, but subsequently many states began to collect data on all subjects tested. Blood lead tests were conducted primarily in response to occupational exposure, but in some cases stemmed from non-occupational exposure (see below for more details). ABLES coverage increased from 4 states in 1987 to 41 states in 2012 (Roscoe *et al.* 2002). We obtained data from 11 state ABLES programs: Connecticut, California, Ohio, Minnesota, Iowa, Pennsylvania, New York, New Jersey, Wisconsin, Michigan and Massachusetts, from their year of first participation until 2008. We excluded everyone who was tested after 2005, to avoid short follow-up times. We also excluded any subjects missing information on date of birth, test date, or blood lead levels. We categorized each blood lead level reading into 1 of 4 categories, namely <5 µg/dl, 5 to <25 µg/dl, 25 to <40 µg/dl, and 40+ µg/dl. These categories <25, 25-40, and 40+ have been traditionally used to categorize occupational blood lead levels, while the lowest category, 5 µg/dl was considered equivalent to non-occupational US blood lead levels. Blood lead category for each individual was defined as the highest category ever achieved.

We first selected all subjects from the states who had ever had a blood lead level reading in categories 3 or 4. We then selected an equal number of people from categories

1 and 2 (50% from each category), stratified by state. Three states (Wisconsin, Michigan and Massachusetts) opted to do their own data processing and matching with the National Death Index (NDI). They followed the same selection pattern, but independently submitted data to NDI and provided de-identified data.

Finally, we restricted our analytic cohort to males, because females represented only 7% of the deaths and were highly concentrated in the lowest blood lead category, which is likely to have included a higher percentage of individuals tested for non-occupational reasons, especially among women tested for lead during pregnancy (personal communication, Susan Payne, California ABLES, May 2013).

We further excluded all people who were tested for the first time after the age of 70 years or before the age of 18 years, as these were unlikely to be occupational exposures (these numbered only a few hundred people), as we wished to the highest extent possible to analyze an occupationally-exposed cohort. We also excluded a few blood tests with a blood lead level greater than 250µg/dl, as these values were considered implausible (n=65). We then further searched for duplicate records across states using combinations of SSN, last name and date of birth, and found 2300 duplicate records with tests in different states. These were assigned the same identity number across states.

We used name, date of birth, gender, race (when available) and SSN (when available) for matching with the NDI database through the end of 2010, to obtain data on date and cause of death (underlying and multiple). We used the probabilistic matching data from NDI to determine a match, and used a standard NIOSH algorithm to determine NDI matches. This ensured uniformity in determining an NDI match, whether the states submitted their data to NDI (due to confidentiality restrictions) and then supplied de-

identified data to the PI, or whether the PI received identifiable data from the states and submitted them to NDI. Ancillary data on states where the test took place was useful in resolving some records where people have common names and there are several matches on name and date of birth, as detailed below. Due to confidentiality issues, some states (~15% of the cohort), namely Wisconsin, Massachusetts and Michigan, sent their data independently to the NDI and USRDS. We received de-identified data for these observations, preventing further follow-up with NDI. Thus, for these three states that independently sent their data, NDI follow-up ended in 2009. For the remaining states, we further submitted data to the NDI for follow-up information for years 2009 and 2010. This was done to increase the number of events in our cohort. To determine if a match with the NDI was a true match from amongst the multiple matches reported by NDI, we only selected those who were assigned a status code of 1 by NDI, indicating a high probability of a match. If a person's last blood lead date was after their date of death, then the match was false and we dropped all information received from NDI, i.e. these subjects were considered as alive. If there were multiple matches with status code 1, we selected the one the NDI reported as an exact match. If there was no exact match, we sorted all the status codes=1 by probability score. If highest probability score was ≥ 40 and state of death was the same as the state where a subject was tested, then we selected that observation. If there were multiple matches meeting this criterion, then we selected the one with the higher probability score of match. In rare instances, when we were unable to select a match based on the above criteria, we dropped those subjects entirely from the final dataset to avoid misclassification of outcome.

After the above exclusions, we had a final analytic dataset with 58,368 unique subjects. About half the subjects (49%) had a single blood test, while the remainder had a median of four. Considering each blood lead test an observation, we had a total of 283,270 observations.

Analyses

The NIOSH Life Table Analysis System (NIOSH, LTAS, Version 3.0) was used to calculate person-years of exposure and rates of death for the cohort, and then compared these rates with those of the US population via standardized mortality rations (SMRs), adjusted for age, race, sex, and calendar time (Robinson *et al.* 2006, Schubauer-Berigan *et al.* 2011). SMRs are calculated for 92 specific causes of death. National rates were used, rather than 11 different state rates, for convenience and because mortality rates in these 11 states (including the large states of Pennsylvania, Ohio, New York, New Jersey, and California) as a whole tend to reflect overall US rates. Life table analyses used the NIOSH life table system (LTAS) for personal computers 311, which in its updated version has 119 separate death categories for multiple causes (including all the categories of *a-priori* interest) and also has state comparison rates for these death categories. The NIOSH program covers the transition from the ninth to the tenth ICD revision (which occurred in 1999). The NIOSH program used the ICD code at time of death and allocated deaths in the appropriate death categories across revisions 265.

NIOSH LTAS requires race gender and race (white vs. non-white) as stratification variables in the analysis. A small number of subjects missing gender (0.2%, n=116) were classified as male. Large numbers of subjects (69%) were missing race and were

classified as white. Among those with known race, 80% were white. Hence, approximately 12% of non-whites in our study were likely to have been misclassified as white, potentially causing some bias in our results.

We calculated SMRs for all blood lead categories (1 through 4) and all categories combined, as well as stratified by time since first exposure (0-5, 5-10, 10+ years).

Person-time at risk began at time of first blood test for everyone. All person-years were assigned to the highest blood level; most people did not change blood lead category. We considered an analysis using a time-dependent categorization of blood lead level, where subjects could change categories across time, but as we did not have complete blood lead histories, we felt such an analysis was not justified.

In internal analyses, we calculated Standardized Rate Ratios (SRRs) using Poisson models (log person-years offset, with scaled standard errors to adjust for over-dispersion), adjusted for gender, race, age category, and calendar period, and comparing each lead category (5 to <25 µg/dl, 25 to <40 µg/dl, and 40+ µg/dl) to the reference category (<5 µg/dl) with SAS version 9.3 (SAS Institute, Cary, NC). Person-time and events in these analyses were grouped in 5 year age and calendar time periods, although age categories below age 50 were generally collapsed for Poisson regression, as few deaths from chronic disease occur prior to age 50, making models unstable. In some instances, when the cause of death was rare, further collapsing of 5 year age and calendar time periods was also required. We also conducted a trend test in Poisson regression by assigning median values of highest blood lead level for each category as the category median.

We also ran several sensitivity analyses. To possibly reduce misclassification of exposure status among people who changed BL categories, we conducted the same analysis on a subset of the people (67%) who did not change BL categories. To determine if absence of SSN (which might have affect NDI matching resulting in misclassification of outcome) would affect the results, we conducted an analysis on the subset of the people with SSN (26%). We also further divided our blood lead category 4 based on its median into very high and extremely high categories (cut-point was 50µg/dl), and thus obtained 5 categories of exposure, namely, <5 µg/dl, 5 to <25 µg/dl, 25 to <40 µg/dl, 40-<50 µg/dl and 50+ µg/dl.

RESULTS

Table 4.1 provides descriptive information about the cohort. There were 58,368 male subjects. The median years of follow-up in the cohort were 12 years (increasing from 6.4 years in lowest blood lead category to 17.1 years in highest). There were 3,337 deaths in the cohort. In general, people in the highest category compared to the lowest category tended to have longer follow-up, more complete information on race, and higher median number of blood lead tests, more complete information on SSN, earlier birth year, and higher number of deaths.

We did not have data on occupation or industry. However, we did have data from California, one of our largest states (10,529 subjects), on whether a blood test was occupational or non-occupational (e.g., from exposure to lead at a shooting range, or lead paint in a residence). Overall, 72% of our California subjects had such data. Of these,

only 2% were non-occupational. People in the highest blood lead category (82%) tended to have more complete information on occupation as compared to the lowest category (48%).

NIOSH has collected data on industry for a limited number of ABLES subjects (n=6,999) (NIOSH, 2008), all in lead categories 3 and 4 (25-49 µg/dl, 40+ µg/dl) (Centers for Disease Control and Prevention 2011). Of these 62% were in manufacturing, 10% in construction, 7% in metal mining, 1% in trade (scrap and waste materials), and 20% were in other industries or data were unavailable. Considering more specific industry categories, the largest groups worked with storage batteries (production or renovation) (43%), painting/paper-hanging (9%), and secondary smelting (9%).

Table 4.2 displays the distribution of the cohort by state and category. Two states, Massachusetts and New York, did not collect blood lead information on people with blood lead levels below 5µg/dl.

About half of the cohort (49%) had only one blood lead test, while another 18% had more than one but did not change category. Another 24% changed category over blood tests, but by only one category. Only 7% of the cohort changed blood lead category by more than one BL level.

Table 4.3 shows the SMRs for causes of *a priori* interest, as well as some smoking-related causes. A healthy worker effect (SMRs less than 1.0) is evident in this cohort, with an all cause SMR of 0.69 (0.66-0.71), and SMRs at or below 1.00 for all specific causes. The only significant (at the p=0.05 level) excesses were noted in the highest lead category (40+ µg/dl), for lung cancer (SMR= 1.20, 95 CI 1.03, 1.39) and laryngeal cancer (SMR= 2.11, 95% CI 1.05, 3.77). Other smoking-related causes in this

blood lead category were not in excess (COPD, ischaemic heart disease, esophagus, stroke), suggesting that smoking alone did not account for the lung and larynx cancer excesses. It should be noted that the number of deaths in this high blood lead category were limited for other cancer outcomes of interest (brain n=11, kidney n=9, stomach n=10). However, the lack of excess in the high blood lead categories for causes associated with blood pressure (stroke, ischemic heart disease, chronic renal disease) did not suggest a major effect of lead on these outcomes.

Table 4.4 shows the internal comparisons via Poisson regression for the outcomes in Table 4.3, with the exception of bladder and brain cancer where there were no deaths in the referent group (blood lead<5 µg/dl). Positive linear trends were found for all causes ($p=0.0001$), lung cancer ($p<0.0001$), COPD ($p=0.02$), ischemic heart disease ($p<0.0001$), and chronic renal disease ($p=0.04$). Some of these positive trends are probably due to the decrease in the healthy worker effect with increased follow-up time, given that follow-up time increased with higher blood lead category (Li *et al.* 1999, Baillargeon 2001).

Table 4.5 shows SMRs for blood lead categories 3 and 4 (25-39 µg/dl, 40+ µg/dl), for selected outcomes with sufficient numbers of deaths, for those with more 20+ years follow-up. The healthy worker effect is less strong (SMR 0.79), while the overall lung cancer SMR is 1.17, increasing to 1.35 (0.92-1.90, 32 deaths) in the highest blood lead category. There is still no suggested increase for those with the highest blood lead for stroke or heart disease.

We conducted several sensitivity analyses, focused on the lung cancer finding. The lung cancer SMR for those who had SSNs (26% of the cohort, those least likely to

incorrectly match with NDI data) was increased to 1.17 (1.01-1.34, 204 deaths), as was the lung cancer SMR for those in the highest blood lead category (SMR 1.42, .17-1.70, 115 deaths). Those who stayed in the same blood lead category (67% of the cohort) had an overall lung cancer SMR of 0.83 (226 deaths), but in the highest blood lead category the SMR was 1.40 (1.08-1.79, 64 deaths). We also looked at lung cancer results after dividing the highest blood lead category into two. The lung cancer SMR for those between 40-<50 μg/dl was 1.13 (0.90-1.39, 87 deaths), increasing to 1.28 (1.02-1.58, 87 deaths) for the group with their highest blood lead ≥50 μg/dl.

DISCUSSION

The strongest associations between lead exposure by category and cancer were for lung cancer in both external and internal analyses, with a modest excess in the highest blood category. There is also some suggestion of an increase of larynx cancer in those with the highest blood lead, but based on small numbers. No other *a-priori* cancers appear in excess, although numbers were small for many of them. Neither stroke, heart disease, nor kidney disease appear to be in excess in the highest blood lead category.

In 2004, both the International Agency for Research on Cancer(IARC) (International Agency for Research on Cancer 2006) and National Toxicology Program (National Toxicology Program 2004) concluded that lead was a probable human carcinogen, based primarily on lung and stomach cancer, with some suggestion of an effect for kidney and brain cancer. The most informative human epidemiology regarding cancer among those with high BL levels comes from seven cohort studies of occupationally exposed cohorts (Fanning 1988, Steenland *et al.* 1992, Anttila *et al.* 1995,

Gerhardsson *et al.* 1995, Lundstrom *et al.* 1997, Wong *et al.* 2000, Carta *et al.* 2005), although these studies are all mortality rather than incidence studies.

Our results provide further support to the thesis that there is a causal association of lead exposure in subsequent development of lung cancer. Our finding of excess larynx cancer is novel. However, we were unable to find associations with stomach, kidney and brain cancer, of a priori interest due to prior studies (IARC 2006). This is possibly due to the relatively young age of the cohort, and the small number of deaths from these causes.

Lead exposure has been associated with modest increases in blood pressure. A meta-analysis of 31 studies by Nawrot et al. 2002 found that most showed a positive association between blood lead and blood pressure after controlling for age, and a doubling of blood lead was associated with a 1.0 mm rise in systolic pressure (95% CI 0.5-1.4), and a 0.6 mm Hg increase in diastolic pressure (95% CI 0.4-0.8).

Increased blood pressure is a risk factor for stroke. However, there is limited information on stroke due to lead exposure. Of the seven key occupational cohort studies cited above, five have information on stroke, and of these, four show some indication of an excess for exposed workers, or sub-sets of exposed workers, compared with low or non-exposed populations; however, confidence intervals for all associations included the null value l. For example, Steenland *et al.* (1992) (Steenland *et al.* 1992), in a study of 1,990 smelter workers, found an elevation for workers with 20 or more years employment (SMR 1.41, 95% CI 0.95-1.85), 26 deaths). Overall, the epidemiological data on the association of lead exposure and cerebrovascular disease is not conclusive. In our present study, we see no increased risk of stroke vs. the US population. However, we did find an increasing risk of mortality from stroke in internal comparisons, where SRRs

increase monotonically with increasing category of lead exposure; however this trend was not statistically significant at the p=0.05 level.

In a review of articles looking at lead and risk of cardiovascular disease (CVD), which is also associated with high blood pressure, Navas-Acien *et al.* (2007) (Navas-Acien *et al.* 2007) found that overall there was insufficient epidemiological data to draw conclusions. In our current study, we found decreased risk of CVD mortality in external comparison, probably as a result of healthy worker effect (HWE), which particularly affects cardiovascular disease. People may develop CVD early on, while still working, while with other diseases like cancer, onset is usually much later in life, usually after retirement. While the healthy worker effect persists for all BL categories in the SMR results for IHD, there is an increasing SMR trend across categories. In internal comparisons, we found statistically significant positive trend in heart disease mortality with increased BL category (p-trend<0.0001). However, this result must be interpreted with caution, given the different lengths of follow-up period by BL categories, and given that the HWE tends to diminish with increased length of follow-up as the cohort ages and is followed past retirement (Checkoway H 2004). Further, follow-up of our cohort will help clarify the heart disease findings.

Very high levels of lead in the body are known to result in kidney failure (U.S. Environmental Protection Agency 2005), but effects at low levels are less clear. Multiple studies have demonstrated the worsening of kidney function (serum creatinine, decreased creatinine clearance) among those exposed to lead, even at low doses of lead exposure (Chia *et al.* 1995, Lin *et al.* 2001, Lin *et al.* 2003, Weaver *et al.* 2003, Tsaih *et al.* 2004, Lin *et al.* 2006, Weaver *et al.* 2009). A recent (2006) comprehensive review of lead-

related nephrotoxicity (Ekong *et al.* 2006) concluded that lead contributes to nephrotoxicity, even at blood lead levels below 5 µg/dl, especially in people with other illnesses, such as hypertension and diabetes. However, it is not known whether such nephrotoxicity is severe enough to lead to chronic renal disease. In our study, in spite of small numbers, in internal comparisons, we found a significant monotonic increase in mortality due to non-malignant kidney disease (p-trend=0.04). However, there were no significant associations between any category of lead exposure and mortality due to kidney disease, in relation to the US population.

Our study has a number of limitations. An important one is the overall healthy worker effect in this cohort with relatively short follow-up, in which only about 6% have died. Furthermore, internal comparisons between those with low blood lead and those with higher blood lead are made difficult by the much higher length of follow-up for the higher blood lead categories, for which the healthy worker effect would be expected to be correspondingly less. This is especially true for non-malignant causes, which are more susceptible to the healthy worker effect. A further limitation is absence of work history, and limited data on blood lead levels over time. While we are reasonably confident that most of those in our cohort were exposed occupationally, we do not know when lead exposure began, so that analyses by true latency are not possible. Additionally, we do not have data on smoking, a potential confounder for our principal findings of interest, lung cancer and larynx cancer. However, apart from lung and larynx cancer, most smoking-related diseases show no excess in the highest blood lead category (i.e., bladder cancer,

heart disease, COPD, esophageal cancer), suggesting confounding by unmeasured smoking is unlikely to explain the positive associations.

Another limitation is the lack of SSNs on 75% of our cohort, and the potential for misclassification of outcome when matching to NDI on name, gender, and date of birth. However, previous work by Williams *et al.* (1992) (Williams *et al.* 1992) using subjects known to be dead or alive, has shown that with first name, last name, and date of birth, investigators can attain a 92% sensitivity (detection by NDI of known dead) and a 92% specificity using NDI (non- matching in NDI for known to be alive). This work was done having only the first initial of the first name, and Williams *et al.* (1992) (Williams *et al.* 1992) suggest that specificity will be increased without loss of sensitivity by having the full first name, as we have in ABLES. Use of name and date of birth have also been shown to be effective for NDI by earlier authors (Stampfer *et al.* 1984). NDI costs are the largest costs in the budget for this project. The only other potential method of ascertaining vital status (but not cause of death) for this population is the Social Security Administration (SSA); however, the SSA requires SSN (www.ssa.gov/policy/about/epidemiology.html).

Confounding by state is another possibility, as some states may have higher rates of disease of interest, and in turn workers in some states may have had higher lead levels. We used national rates for our SMR and SRR analyses.

Non-whites (African-Americans) have higher rates of some diseases, including some of *a priori* interest (e.g. lung cancer, stroke), and may have had higher levels of lead exposure. We are missing data on race for 69% of our cohort. Our data on race

among the 31% not missing race, indicates that non-whites increase from 15% non-white in lowest BLL category (category 1), to 18% non-white in category 4). Such differences are minimal, suggesting that race can be responsible for only minimal confounding for disease, which is not very strongly associated with race. In the mortality analysis, we have opted for assigning white race to all those missing race. For most diseases not strongly related to race, this affects our estimates of lead effect only minimally. If we assume the percent non-white among those missing race about the same as the 31% for whom we have race (i.e., 17%), then we have misclassified 69%*17% or 12 % of the population as white, when they are in fact non-white. For lung cancer, a disease of *a priori* interest, which is perhaps most strongly related to smoking, non-white males have rates which are 10% higher than white. Among those with known race, 80% were white. We can presume that most non-whites in our study population were black, as is the case for non-whites in the US as a whole. For lung cancer, the black male rate over the last 20 years has been 87/100,000, compared to a white rate of 68/100,000, and the combined male rate has been 69/100,000. In our study population, we assumed overall a population of 6% non-white, where it is likely that the true non-white population was 20%. Combining these data, it is likely that we have underestimated our US population lung cancer rate by about 4%, and hence we have over-estimated our lung cancer SMRs by about 4%. This is only a small amount, and our basic conclusions regarding lung cancer are unchanged.

It should also be noted that our study has a number of strengths, the most important of which is a large study population with documented blood lead levels.

Furthermore, we studied a large number of subjects with documented levels ≥40 μg/dl, which is the level that OSHA considers acceptably safe for workers exposed occupationally.

CONCLUSION

We found some evidence of increased risk with higher lead exposure for lung cancer and larynx cancer. In this cohort, we also found a positive trend by increasing lead category for ischaemic heart disease. However, the length of follow-up increases greatly with BL category, making it difficult to interpret increasing trends of risk by increasing BL categories. Data are also limited by lack of work history, no data on smoking, and small numbers of deaths in some categories. Since this is a relatively young cohort with short follow-up and few deaths, re-examination of these associations after a few years may shed more light on the chronic health effects of adult lead exposure.

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Table 4.1: Demographics of the Cohort (N=58,368)

		Highest Lead Category Achieved				
Characteristics		**1**	**2**	**3**	**4**	**Total**
		0-<5 µg/dl	**5-<25 µg/dl**	**25-<40 µg/dl**	**40+ µg/dl**	
Total		6,848 (11.7%)	18,650 (31.9%)	21,448 (36.7%)	11,422 (19.6%)	58,368
Median Years of Follow-up		6.4	9.9	14.2	17.1	12
Age at First Test		40.7 (12.2)	39.9 (11.7)	37.9 (11.4)	38.3 (11.5)	38.9 (11.7)
Race	**White**	1,448 (21.1%)	2,356 (12.6%)	6,246 (29.1%)	4,339 (38.0%)	14,389 (24.7%)
	Non-White	252 (3.7%)	558 (3.0%)	1,673 (7.8%)	1,200 (10.5%)	3,683 (6.3%)
	Missing/Unknown	5,148 (75.2%)	15,736 (84.4%)	13,529 (63.1%)	5,883 (51.5%)	40,296 (69.0%)
Median number of Observations in those with > 1		2	3	4	6	4
% with Single Observations		6,124 (89.4%)	12,739 (68.3%)	7,786 (36.3%)	1,940 (16.9%)	28,589 (48.9%)
Mean Highest Blood Lead Level		2.5 (1.1)	12.9 (5.5)	30.8 (4.2)	52.0 (15.1)	25.9 (17.9)
% with SSN (for matching)-Overall#		611 (8.9%)	2,084 (11.2%)	7,664 (35.7%)	4,883 (42.8%)	15,242 (26.1%)
Median Year of Birth		1962	1961	1959	1955	1959
Median Year of Death		2006	2005	2004	2003	2004
Number Dead		173 (2.5%)	635 (3.4%)	1,301 (6.1%)	1,228 (10.8%)	3,337 (5.7%)

Some states like WI, MI, MA and PA sent their own data and sent us de-identified data, without SSN, so these percentages are underestimates
*we excluded any record with a blood lead level greater than 250 as there were considered to be implausible. (N=26)

Table 4.2 Number of people by state and lead category (n=58,368)

	Highest Lead Category Achieved				
STATE	**1**	**2**	**3**	**4**	**TOTAL**
	0-<5 µg/dl	**5-<25 µg/dl**	**25-<40 µg/dl**	**40+ µg/dl**	
TOTAL	6,848	18,650	21,448	11,422	58,368
California	1,937 (28%)*	2,673 (14%)	3,917 (18%)	2,002 (17%)	10,529 (18%)
Connecticut	385 (6%)	582 (3%)	895 (4%)	343 (3%)	2,205 (4%)
Iowa	373 (5%)	391 (2%)	572 (3%)	329 (3%)	1,665 (3%)
Massachusetts		2,383 (13%)	2,531 (12%)	1,284 (11%)	6,198 (11%)
Michigan	292 (4%)	471 (2%)	790 (4%)	274 (2%)	1,827 (3%)
Minnesota	190 (3%)	335 (2%)	543 (2%)	212 (2%)	1,280 (2%)
New Jersey	1,598 (23%)	1,190 (6%)	2,098 (10%)	1,412 (12%)	6,298 (11%)
New York		7,690 (41%)	5,493 (26%)	3,786 (33%)	16,969 (29%)
Ohio	1,129 (16%)	1,524 (8%)	2,136 (10%)	1,094 (10%)	5,883 (10%)
Pennsylvania	336 (5%)	536 (3%)	1,005 (5%)	167 (1%)	2,044 (3%)
Wisconsin	608 (9%)	875 (5%)	1,468 (7%)	519 (4%)	3,470 (6%)

- Column percentages

Table 4.3 Standardized Mortality Ratios (SMR) and 95% confidence intervals assessing the effect of lead category of exposure on mortality due to various causes in the cohort (n=58,368)

Cause of Mortality		Highest Lead Category Achieved									
		1		2		3		4		Overall	
		0-<5 µg/dl		5-<25 µg/dl		25-<40 µg/dl		40+ µg/dl			
		N	SMR (95% CI)	N	SMR (95% CI)	N	SMR (95% CI)	N	SMR (95% CI)	N	SMR (95% CI)
All Causes		173	0.63 (0.54, 0.73)	635	0.59 (0.55, 0.64)	1301	0.66 (0.63, 0.70)	1228	0.80 (0.75, 0.84)	3337	0.69 (0.66, 0.71)
Cancer	Lung, Trachea and Bronchus	10	0.42 (0.20, 0.77)	54	0.56 (0.42, 0.74)	144	0.81 (0.68, 0.95)	174	1.20 (1.03, 1.39)	382	0.86 (0.78, 0.95)
	Brain	0	0.0 (0.0, 1.26)	8	0.71 (0.31, 1.40)	11	0.59 (0.30, 1.06)	11	0.83 (0.41, 1.49)	30	0.65 (0.44, 0.93)
	Kidney	1	0.42 (0.01, 2.35)	9	0.96 (0.44, 1.83)	9	0.55 (0.25, 1.05)	9	0.72 (0.33, 1.37)	28	0.69 (0.46, 1.00)
	Stomach	2	1.19 (0.14, 4.32)	2	0.3 (0.04, 1.08)	9	0.69 (0.31, 1.30)	10	0.92 (0.44, 1.69)	23	0.71 (0.45, 1.07)
	Oesophagus ‡	2	0.59 (0.07, 2.14)	11	0.85 (0.42, 1.52)	13	0.59 (0.31, 1.01)	11	0.65 (0.32, 1.16)	37	0.67 (0.47, 0.92)
	Larynx‡	1	1.15 (0.03, 6.40)	2	0.58 (0.07, 2.10)	2	0.31 (0.04, 1.12)	11	2.11 (1.05, 3.77)	16	1.00 (0.57, 1.63)
	Bladder‡	0	0.00 (0.00,	6	0.90 (0.33,	9	0.74 (0.34,	7	0.70 (0.28,	22	0.72 (0.45,

			2.20)		1.96)		1.41)		1.45)		1.10)
Stroke		4	0.48 (0.13, 1.24)	18	0.54 (0.32, 0.86)	54	0.79 (0.59, 1.03)	47	0.79 (0.58, 1.05)	123	0.73 (0.60, 0.87)
Chronic Obstructive Pulmonary Disease‡		10	1.04 (0.50, 1.92)	12	0.31 (0.16, 0.54)	45	0.61 (0.45, 0.82)	53	0.86 (0.64, 1.12)	120	0.65 (0.54, 0.78)
Cardiovascular Disease	**Ischaemic Heart Disease**	21	0.44 (0.27, 0.67)	95	0.49 (0.39, 0.60)	230	0.62 (0.54, 0.70)	223	0.72 (0.63, 0.82)	569	0.61 (0.56, 0.67)
	Hypertension with Heart Disease	3	0.81 (0.17, 2.36)	10	0.73 (0.35, 1.35)	13	0.52 (0.28, 0.89)	21	1.08 (0.67, 1.65)	47	0.76 (0.56, 1.01)
	Hypertension without Heart Disease	2	1.43 (0.17, 5.17)	6	1.14 (0.42, 2.49)	4	0.39 (0.11, 1.00)	7	0.83 (0.33, 1.70)	19	0.75 (0.45, 1.17)
	Any Hypertension	5	0.98 (0.12, 1.84)	16	0.85 (0.43, 1.26)	17	0.48 (0.25, 0.71)	28	1.00 (0.63, 1.37)	66	0.76 (0.57, 0.94)
Chronic Renal Disease		2	0.78 (0.09, 2.82)	3	0.31 (0.06, 0.89)	10	0.52 (0.25, 0.96)	16	1.01 (0.58, 1.64)	31	0.65 (0.44, 0.93)
Any Hypertension or Chronic Renal Disease		7	0.91 (0.24, 1.59)	19	0.66 (0.36, 0.96)	27	0.50 (0.31, 0.68)	44	0.98 (0.69, 1.28)	97	0.71 (0.57, 0.86)

‡ Smoking related diseases

Table 4.4 Standardized Rate Ratios and 95% confidence intervals by Lead Category using Poisson Models. (n=58,368)

Cause of Mortality[b]		Highest Lead Category Achieved								p-value for Test of Trend[a]
		1		2		3		4		
		0-<5 µg/dl		5-<25 µg/dl		25-<40 µg/dl		40+ µg/dl		
		N	SRR (95% CI)	N	SRR (95% CI)	N	SRR (95% CI)	N	SRR (95% CI)	
All Causes		173	Ref	635	0.96 (0.68, 1.34)	1301	1.11 (0.81, 1.54)	1228	1.41 (1.01, 1.96)	0.0001
Cancer	**Lung, Trachea and Bronchus**	10	Ref	54	1.34 (0.79, 2.26)	144	1.88 (1.14, 3.10)	174	2.79 (1.69, 4.61)	<0.0001
	Kidney	1	Ref	9	2.41 (0.62, 9.46)	9	1.31 (0.33,5 .20)	9	1.70 (0.42, 6.83)	0.62
	Stomach	2	Ref	2	0.24 (0.04, 1.34)	9	0.52 (0.13, 2.04)	10	0.64 (0.16, 2.60)	0.49
	Oesophagus[‡]	2	Ref	11	1.54 (0.76, 3.11)	13	1.15 (0.57, 2.32)	11	1.39 (0.68, 2.85)	0.99
	Larynx[‡]	1	Ref	2	0.54 (0.05, 5.97)	2	0.36 (0.03, 4.01)	11	2.96 (0.37, 23.62)	0.14
Stroke		4	Ref	18	1.12 (0.32, 3.94)	54	1.76 (0.54, 5.76)	47	1.88 (0.57, 6.28)	0.095
Chronic Obstructive Pulmonary Disease[‡]		10	Ref	12	0.30 (0.15, 0.61)	45	0.59 (0.33, 1.05)	53	0.85 (0.47, 1.53)	0.02
Cardiovascular Disease	**Ischaemic Heart Disease**	21	Ref	95	1.13 (0.78, 1.66)	230	1.46 (1.02, 2.10)	223	1.77 (1.23, 2.56)	<0.0001
	Hypertension with Heart Disease	3	Ref	10	0.92 (0.01, 130.42)	13	0.76 (0.01, 99.08)	21	1.77 (0.02, 204.73)	0.67

	Hypertension without Heart Disease	2	Ref	6	0.73 (0.12, 4.45)	4	0.28 (0.04, 1.94)	7	0.64 (0.10, 3.99)	0.64
	Any Hypertension	5	Ref	16	0.85 (0.04, 18.84)	17	0.56 (0.02, 12.52)	28	1.29 (0.06, 26.13)	0.72
Chronic Renal Disease		2	Ref	3	0.39 (0.09, 1.77)	10	0.73 (0.20, 2.64)	16	1.52 (0.43, 5.38)	0.04
Any Hypertension or Chronic Renal Disease		7	Ref	19	0.72 (0.11, 4.81)	27	0.61 (0.10, 3.85)	44	1.36 (0.23, 8.16)	0.35

[a] P-value is for linear trend test by assigning medians for highest blood lead in each category.

[b] Bladder cancer had too few numbers to run a trend test on and so is not reported in this table.

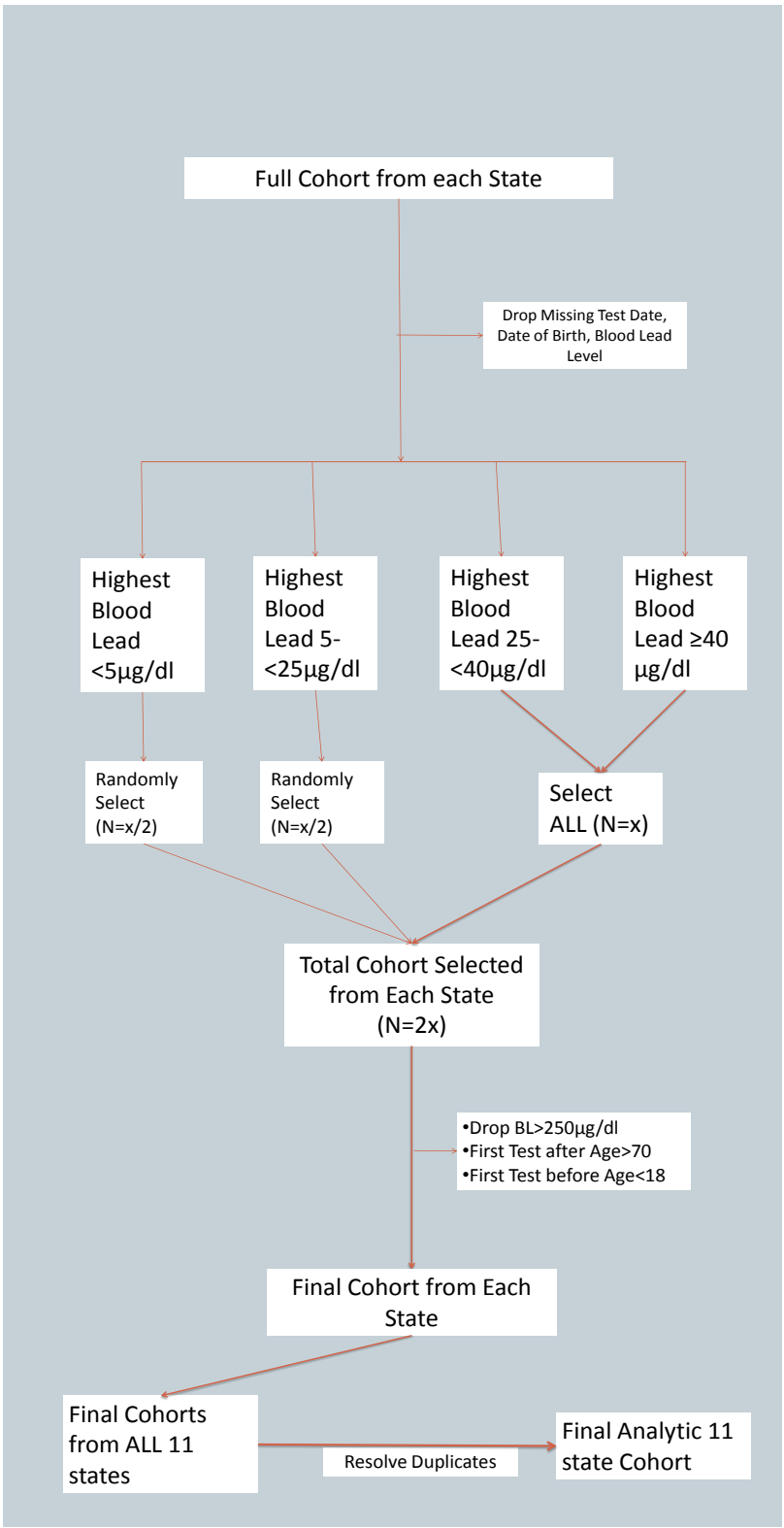
[‡] Smoking related diseases

Table 4.5 Standardized Mortality Ratios and 95% Confidence Intervals among those with more than 20 years follow-up.

	Blood lead 25-<40		Blood lead 40+		All	
	N	**SMR (95% CI)**	**N**	**SMR (95% CI)**	**N**	**SMR (95% CI)**
All Causes	92	0.73 (0.59, 0.90)	213	0.83 (0.72, 0.95)	305	0.79 (0.71, 0.89)
Lung Cancer	10	0.83 (0.40, 1.52)	32	1.35 (0.92, 1.90)	42	1.17 (0.84, 1.58)
Stroke	6	1.06 (0.39, 2.31)	8	0.66 (0.28, 1.30)	14	0.78 (0.43, 1.31)
Chronic Obstructive Pulmonary Disease‡	3	0.43 (0.09, 1.27)	16	1.14 (0.65, 1.85)	19	0.90 (0.54, 1.41)
Ischaemic Heart Disease	18	0.72 (0.43, 1.14)	45	0.86 (0.63, 1.16)	63	0.81 (0.63, 1.04)

‡Smoking related diseases

Figure 4.1 Cohort Selection Flowchart



CHAPTER 5. Lead exposure and incident End Stage Renal Disease (ESRD) among participants in a lead surveillance program

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Key Words: ESRD, Lead, Kidney disease, Occupational Exposure, ABLES

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Abbreviations: ABLES: Adult Blood Lead Epidemiology & Surveillance

ESRD: End Stage Renal Disease

BL: Blood Lead

ABSTRACT

Background: Very high levels of lead can cause renal failure, but data are sparse on renal effects at lower levels.

Methods: We studied men who were part of a NIOSH-sponsored occupational lead surveillance system in 11 states. Incident end-stage renal disease (ESRD) was determined via matching with the US Renal Data System (URDS), and analyzed in external and internal analyses. BL categories were 0-<5, 5-<25, 25-<40, and 40-<51, and ≥ 51μg/dl, defined by highest blood lead test. Data on race was available for all ESRD cases, but was missing for 69% of the source cohort based on state data. We conducted two analyses, one restricted to the subset of the cohort with race information (31%, 108 ESRD cases), and another of the whole cohort imputing race when missing.

Results: There were 58,307 men with a median 12 years of follow-up and 302 incident ESRD cases. Most workers (67%) had only one BL test, while the remainder had a median of four. In analyses restricted those with race information, the ESRD standardized rate ratio (SIR) for the cohort vs. the US was 1.08 (0.89-1.31). Those in the highest BL category had a SIR of 1.47 (0.98-2.11) (test for trend across BL categories, p=0.08), increasing to 1.56 (1.02-2.29) for those with 5+ years follow-up. For the entire cohort (race imputed for non-cases), the overall SIR was 0.92 (0.82-1.03) increasing to 1.36 (0.99-1.73) in the highest BL category. RRs across BL categories (were 1.0 (categories 1 and 2 combined), 0.80, 1.03, and 1.59; the test for trend (p=0.003).

Conclusion: We found ESRD incidence for those in the highest BL category (51+μg/dl).Data are limited by the lack of detailed work history and reliance on only a few blood lead tests per person to estimate level of exposure, and lack of information on

risk factors, such as hypertension and diabetes.

INTRODUCTION

High levels of lead exposure have historically been shown to lead to an increased risk of acute renal failure (U.S. Environmental Protection Agency 2006). There are some data from occupational cohort mortality studies indicating that workers exposed to high lead levels may have increased chronic renal disease (Henderson 1955, Cooper *et al.* 1985, Steenland *et al.* 1990). More recent data suggests that lead can impair kidney function even at low levels (Staessen *et al.* 1992, Payton *et al.* 1994, Chia *et al.* 1995, Staessen 1995, Staessen *et al.* 1995, Kim *et al.* 1996, Lin *et al.* 2001a, Lin *et al.* 2001b, Weaver *et al.* 2003, Tsaih *et al.* 2004, Lin *et al.* 2006, Weaver *et al.* 2009).

Other potential risk factors for end-stage renal disease (ESRD) include hypertension, race and diabetes. Non-whites in the US are known to have much higher ESRD rates than whites, presumably due in part to the higher prevalence of hypertension in this group, which is primarily composed of African-Americans. For example, age and gender-adjusted SRD incidence rates (per 100,000) for African-Americans and whites in 2010 in the US were 92.4 and 27.5, respectively (http://www.usrds.org/reference.aspx, accessed June 9, 2013). These factors must be taken into account while comparing risk of ESRD among lead exposed people.

Here we have studied the incidence of ESRD among men tested for blood lead in 11 states who were part of an occupational blood lead surveillance program, the Adult Blood Lead Surveillance (ABLES) program, sponsored by the National Institute for Occupational Safety and Health (NIOSH).

METHODS

Data Sources/Study Participants

The Adult Blood Lead Surveillance (ABLES) program, sponsored by the National Institute for Occupational Safety and Health (NIOSH), started collecting state-level data on blood leads exposure in 1987 (Roscoe *et al.* 2002). In participating states, state agencies collected data on all adult subjects with lead tests in any laboratory in the state. Initially, some states gathered data only on those whose blood lead levels exceeded 25μg/dl, but subsequently most states began to collect data on all subjects tested. Blood lead tests were primarily due to occupational exposure, but in some cases, stemmed from non-occupational exposure (see below for more details). ABLES coverage increased from 4 states in 1987 to 41 states in 2012 (Roscoe *et al.* 2002). We obtained data from 11 state ABLES programs: Connecticut, California, Ohio, Minnesota, Iowa, Pennsylvania, New York, New Jersey, Wisconsin, Michigan and Massachusetts from their year of first participation through 2008. We excluded everyone who was tested after 2005, to avoid very short follow-up time. We also excluded any subjects missing information on date of birth, test date, or blood lead levels. We categorized each blood lead level reading into 1 of 5 categories, namely <5 μg/dl, 5 to <25 μg/dl, 25 to <40 μg/dl, 40-<51 μg/dl, and ≥51 μg/dl, or categories 1 through 5, respectively. Categories of <25, 25-40, and 40+ have been traditionally used to categorize occupational blood leads, while the lowest category 5 μg/dl essentially was equivalent to non-occupational US blood lead levels. We further divided the upper category into two groups with approximately equal number of ESRD cases to explore whether there was an excess risk confined to those with particularly high

BLs. If a subject had more than one blood lead test available, blood lead category was defined as the highest category ever achieved.

We first selected everyone from the states who had ever had a blood lead level reading in categories 3 or 4. We then selected an equal number of people from categories 1 and 2 (50% from each category), stratified by state. Three states (Wisconsin, Michigan, and Massachusetts) opted to do their own data processing and matching with the National Death Index (NDI) and the United States Renal Data System (USRDS). They, too, followed the same selection pattern but independently submitted data to NDI and USRDS and sent us de-identified data.

We restricted our analytic cohort to males, because females represented only 9% of ESRD cases and were concentrated more heavily in the lowest blood lead category, which is likely to have included a higher percentage of non-occupational blood leads, especially among women tested for lead during pregnancy (personal communication, Susan Payne, California ABLES, May 2013).

We further excluded all people who were tested for the first time after the age of 70 years or before the age of 18 years, as these were unlikely to be occupational exposures (these numbered only a few hundred people), as we wished to the highest extent possible to analyze an occupationally-exposed cohort. We also excluded a few blood tests with a blood lead level greater than 250µg/dl, as these values were considered implausible (n=65). We then further searched for duplicate records across states using combinations of SSN, last name and date of birth and found 2300 duplicate records with tests in different states. These were then assigned the same identity number across states.

We used name, date of birth, gender, race (when available) and SSN (when available) for matching with the NDI and USRDS databases through the end of 2010, to obtain data on date of death and on ESRD incidence. The end of follow-up was 2009 for the three states which did their own matching (about 15% of the cohort).To determine if a match with the NDI was a true match from amongst the multiple matches reported by NDI, we only selected those who were assigned a status code of 1 by NDI, indicating a high probability of a match. Validation studies have shown that about 95% of all US patients with ESRD are in the USRDS system (1992, USRDS 1992). Incidence rates of ESRD are available since 1973 and currently go through 2007. We used these rates which were extrapolated till 2014. The rates are age, race, sex, and year specific. USRDS used name and date of birth and other variables for matching, analogous to the matching done by NDI. Similar matching of other occupational cohorts has been done in the past (Calvert *et al.* 1997, Steenland *et al.* 2001, Radican *et al.* 2006). Our initial matching with the USRDS gave us follow-up information till 2008. Due to confidentiality issues, some states (~15% of the cohort), namely Wisconsin, Massachusetts and Michigan, sent their data independently to the NDI and USRDS. We received de-identified data for these observations directly from the states, preventing further follow-up with USRDS. In 2012, we sent our dataset to USRDS again for follow-up information till 2010.

In addition to standard list of matching variables, we also requested the USRDS to provide detailed information on the following Core Standard Analysis Files (SAF) data

- Treatment History (RXHIST)
- Medical Evidence (MEDEVID95)
- Medical Evidence (MEDEVID05)
- Death (DEATH)

- Transplant (TX)
- Patients

We lost some ESRD cases (n=60) as their outcomes had occurred (ESRD diagnosis) before or on the same day as their first blood lead level measurement. LTAS dropped these observations from further calculations and analyses. We also lost 1 case as their year of diagnosis was unknown (9999). For USRDS, we accepted all matches as determined by USRDS.

After the above exclusions, we had a final analytic dataset with 58,307 unique subjects, but of these, the majority (69%) was missing data on race.

Adjustment for missing race.

As noted above, non-whites are known to have much higher rates of ESRD incidence than whites in the US. Furthermore, in those with known race based on state data (31% of the cohort), race (white/non-white) was a confounder, in that it was strongly related to ESRD incidence, and also weakly related to BL category (higher BL categories had higher percent non-whites, 18% in the highest t BL category, 15% in the highest). Hence, the fact that 69% of our cohort was missing data on race created potential for bias, although this would be expected to be limited given the relatively weak association between race and BL category among those with known race. Because race was a required field to run the NIOSH life table, we were unable to use LTAS without assigning some value for race (white/non-white) to those missing race. It should be noted, however, that we had race data on all ESRD cases from the USRDS, such that only the non-cases required imputation for race. We chose two strategies to confront the problem of missing race.

First, we conducted an analysis restricted to the 31% (18,057) with known information on race (including 108 ESRD cases arising from this sub-cohort). Second, we imputed race for those missing race in the entire cohort (although not for the 302 ESRD cases arising from the total cohort, for whom we had data on race) via multiple imputation (five imputations), and ran a life table for the entire cohort (five data sets). To do this, we first built an imputation model using logistic regression (in the SAS procedure MI) to predict race (white/non-white) among those with known race. Predictors in this model included year of birth, BL category, state where tested, vital status, year of first lead test, and the presence or absence of ESRD. This model resulted in correct prediction of race for 69% of the observations, incorrect for 30%, and 'tied' data for 2% of observations. The area under the ROC curve was 0.69, indicating moderate success in predicting race by the model, but better than randomly assigning race to those missing it. We then used the regression coefficients from this model (again via SAS PROC MI) to generate five data sets for the entire cohort in which race was imputed for the 69% missing race. Imputation of race for those missing race was done via SAS PROC MI, via five Monte Carlo runs in which the coefficients from the imputation model were assumed to be multivariate normal and a draw was made from this multivariate distribution for each person, generating a predicted probability of race (between 0 and 1) for each subject missing race, and then race was assigned for each individual using a binomial distribution based on each given probability 'p'. This resulted in five imputation data sets. The percentage non-white race in these five data sets varied only slightly, from 18% to 20%, and was similar to those with known race (17% non-white). We then ran life table analyses separately for each of these imputed data sets, and averaged the results

(specifically we averaged the expected ESRD cases, as the observed did not change). We highlight that race was known for all ESRD cases from USRDS, and therefore we did not need to impute race for the cases. The variance of the ensuing summary averaged rate ratio assumed that the observed number of cases was Poisson distributed and that the expected number of cases was invariate. This was not technically true as the expected number of ESRD cases varied across the five life table runs. However, this variance of the expected cases was very small and assumed again that the average expected were invariate.

Analyses

The NIOSH Life Table Analysis System (NIOSH, LTAS, Version 3.0) (Schubauer-Berigan MK 2005) was used to calculate person-years of exposure and rates of ESRD incidence for the cohort, and then to compare these rates with those of the US population via standardized incidence ratios (SIRs), adjusted for age, race, sex, and calendar (Robinson *et al.* 2006, Schubauer-Berigan *et al.* 2011). SIRs were calculated for all ESRD combined, and also calculated for 13 different categories of ESRD, of which the most important (common) are diabetic and hypertensive nephropathy. NIOSH LTAS provides national US ESRD incidence rates, as calculated by the USDRS, stratified by age, gender, race, and calendar-time. NIOSH LTAS requires gender, which is a stratifier in the analysis. A small number of subjects missing gender (0.2%, n=116) were classified as male.

We calculated SIRs for all blood lead categories (1 through 5) and all categories combined, as well as by time since first exposure (0-5, 5+ years). Person-time at risk began at time of first blood test for everyone. All person-years were assigned to the

highest blood level; most people did not change blood lead category. About half of the cohort (49%) had only one blood lead test, while another 18% had more than one but did not change category. Another 24% changed category over blood tests, but by only one category. Only 7% of the cohort changed blood lead category by more than one BL level. We considered an analysis using a time-dependent categorization of blood lead level, where subjects could change categories across time, but as we did not have complete blood lead histories we felt such an analysis not justified. Person-time ended for everyone in 2010, the end of our NDI follow-up.

In internal analyses, we calculated Standardized Rate Ratios using Poisson models (log person-years offset, p-scale option to adjust for over-dispersion), adjusted for gender, race, age category, and calendar period, and comparing each lead category (5 to <25 μg/dl, 25 to <40 μg/dl, 40-<51, and ≥51 μg/dl) to the reference category (<5 μg/dl) with SAS version 9.3 (SAS Institute, Cary, NC). We used national rates, given that our cohort included many large states and the average of state rates was assumed to approximate national rates; an empirical check of all cause death rates across the states included confirmed this assumption). We also conducted a trend test for different diseases in internal analyses via Poisson regression, using age, calendar-time, race-specific rates; Poisson regression results were generated as SRRs (standardized rate ratios) by lead category, and we tested a linear trend in SRRs by assigning median values of highest blood lead level for each category.

RESULTS

Table 5.1 provides descriptive information about the cohort. There were 58,307 male subjects. Forty-nine percent had only one blood test, which the remainder had a median of 4 tests. The median years of follow-up in the cohort were 12 years (increasing from 6.4 years in lowest blood lead category to 17.7 years in highest). There were 3,337 deaths in the cohort. In general, people in the highest category compared to the lowest category tended to have longer follow-up, higher median number of blood lead tests, more complete information on SSN, and earlier birth year.

We did not have data on occupation or industry. However, we did have data from California, one of our largest states (10,529 subjects), on whether a blood test was occupational or non-occupational (e.g., from exposure to lead at a shooting range, or lead paint in a residence). Overall, 72% of our California subjects had such data. Of these, only 2% were non-occupational. People in the highest blood lead category (82%) tended to have more complete information on occupation as compared to the lowest category (48%).

NIOSH has collected data on industry for a limited number of ABLES subjects (n=6,999) (NIOSH, 2008), all in lead categories 3 and 4 (25-49 µg/dl, 40+ µg/dl) (2011). Of these, 62% were in manufacturing, 10% in construction, 7% in metal mining, 1% in trade (scrap and waste materials), and 20% were in other industries or data were unavailable. Considering more specific industry categories, the largest groups worked with storage batteries (production or renovation) (43%), painting/paper-hanging (9%), and secondary smelting (9%).

Table 5.2 displays the distribution of the cohort by state and category. Two states, Massachusetts and New York, did not collect blood lead information on people with blood leads below 5µg/dl.

Table 5.3 shows the results of comparison for incident ESRD in the cohort by lead category using the US population as referent (external comparison), for those for whom we had race data from the states. Overall, there was a slight excess of ESRD (SIR=1.08, 95% CI 0.89-1.31, 108 cases) when compared to the general population. There was some elevation in the highest BL category (SIR 1.47, 95% CI 0.98-2.11), which was more marked for non-whites (SIR 2.12, 1.16-3.56, 14 cases) than whites (SIR 1.14 (0.64-1.89). When restricting the cohort to those with at least 5 years follow-up, the elevation in the highest BL category was more marked (SIR=1.56, 95% CI 1.02-2.29, 26 cases). Combining categories 1 and 2, both of which had a small number of cases, as the referent, the SRRs from Poisson regression across categories 0-24, 25-40, 40-<51, and 51+ were 1.0, 0.87, 1.03, 1.43, with a test for linear trend of p=0.08 (internal comparison). SRRs for 5+ years of follow-up were similar.

Table 5.4 shows the results for the entire cohort using the imputed data for race. Results in Table 5.4 are based on the averages of expected cases across the 5 simulations. The expected did not differ much between simulations. For example, for all lead categories combined, the expected cases range from 325.6-328.8, mean 327.5, variance 1.75. The overall rate ratio was not remarkable (0.92), but again we see an excess in the highest BL category (SIR 1.36 (0.99-1.73). The tendency towards an excess in the highest category was accentuated in those followed for at least 5 years (SIR 1.43 (1.01-.185). For the 5+ years follow-up group, we collapsed BL categories 1 and 2 for a test for

trend, as observed cases were few (n=9) in BL category 1. SRRs across BL categories (again averaging across five imputed data sets) were 1.0 (categories 1 and 2 combined), 0.80, 1.03, and 1.59, with a strong positive trend (p=0.003), based on the elevation in the highest BL category.

DISCUSSION

We have found evidence of increased ESRD incidence in this lead exposed cohort. We found these both in external and internal comparisons for those with their highest blood lead ≥51 μg/dl, i.e., the highest BL category. We found this evidence both in analyses restricted to the 31% of the cohort with known race information available from the states where our data originated, as well as in the entire cohort for which we imputed race where it was missing. Furthermore, in the full cohort, we found a strong positive trend of increased risk with increased BL category (p=0.003) in internal comparisons.

In our full cohort analysis using imputed race data, we did not have to impute data for our ESRD cases, because we obtained these data from the USRDS, independently of the states. We believe these data on race for ESRD cases are likely to be reliable, because of the level of detail of the data collected by the USRDS on all ESRD cases. The availability of race data for cases meant that we had to impute only for non-cases who formed the bulk of our person-time (denominator) data. The availability of race data for our numerators in our cohort ESRD rates meant that uncertainty in the imputation was restricted to the denominators, from which we derived the expected cases to compare to the observed cases in the life table analysis. Our imputation model was only moderately

successful (AUC for the ROC curve 0.69), but nonetheless would be expected to reasonably allocate race, such that our stratification by person-time by imputed race (in five separate runs) would be expected to be a reasonable approximation. It is possible that our finding of an excess of ESRD in the highest blood category reflects the wearing off of the healthy worker effect (HWE) in this largely occupational cohort, given that follow-up time increased for those in the highest BL categories, which would be expected to result in a wearing off of the HWE. However, two points argue against this theory. First, there was little evidence of an overall HWE for the entire cohort (SIR overall of 0.92 in the entire cohort, SMR of 1.08 in the sub-cohort with known race). Second, ESRD typically occurs at older ages after workers have retired, which suggests *a priori* that ESRD would not be expected to be strongly affected by a HWE.

The excess we found in the highest BL category was particularly pronounced among those with more than 5 years follow-up from first blood lead test, which is consistent with the theory that exposures causing chronic diseases typically require some latency period. However, follow-up given our lack of detailed work history, we do not know the true date of initial lead exposure.

The excess in the highest category was more pronounced for non-whites than whites. Non-whites are known to have much higher ESRD rates than whites, presumably due in part to the higher prevalence of hypertension in this group, which is primarily composed of African-Americans. Our finding may imply that high lead exposure exacerbates the already high underlying risk for the non-white group.

Our findings for excess risk in the group with highest BL, although limited, conform to the literature, which indicates that very high blood lead levels can cause acute renal failure (EPA 2005). Therefore, it may not be surprising that the high levels can contribute to chronic renal disease. It should be noted that the highest BL group in our study (7% of the cohort) was composed of those with blood leads of 51µg/dl or more, a level (≥ 50 µg/dl) for which OSHA requires removal of a worker to a lower-exposed job until his/her BL is lowered below 40 µg/dl. Our data re-inforce the need for enforcement of this OSHA standard.

Our results support results from prior occupational studies. Cooper *et al.* (1985) (Cooper *et al.* 1985) found SMRs for chronic kidney disease (nephritis) of 2.22 (95% CI 1.55-3.45) and 2.65 (95% CI 1.14-5.22) in cohorts of battery and lead smelter workers with mean blood leads of 65 µg/dl and 50 µg/dl, respectively. Steenland *et al.* (1992a) (Steenland *et al.* 1992) found an SMR of 1.55 (95% CI 0.66-1.39, 7 deaths) for kidney disease mortality, increasing to 2.79 for workers with 20+ years employment, among smelter workers who had a mean blood lead of 56 µg/dl in the 1970s. Cocco *et al.* (1997) (Cocco *et al.* 1997) found an SMR for genito-urinary disease (ICD 580-608.9) of 1.35 (95% CI 0.74-3.37). Analyses by length of employment found a significant trend of more genito-urinary disease mortality with longer employment (p=0.002), and a borderline trend for the subset of renal failure (p=0.09). On the other hand, Fanning (1988) found a no excess of renal disease (11 deaths) (Fanning 1988). The occupational studies have been limited to mortality, a less sensitive endpoint than morbidity, and subject to misclassification due to inaccuracies determining underlying cause on death certificates. Furthermore, there have been small numbers of deaths in these occupational studies.

In recent years, there has also been increasing evidence of lead's negative effect on renal function (rather than fully developed renal disease) at lower levels of lead exposure than those studies in earlier occupational cohorts. Lead-associated changes of markers, such as by increased serum creatinine, decreased creatinine clearance both indicators of poor glomerular filtration, have been found at low exposure levels in general populations (Staessen *et al.* 1992, Payton *et al.* 1994, Chia *et al.* 1995, Kim *et al.* 1996, Lin *et al.* 2001a, Lin *et al.* 2001b, Weaver *et al.* 2003, Tsaih *et al.* 2004, Lin *et al.* 2006, Weaver *et al.* 2009). Such effects may be early markers of subsequent chronic renal disease. For example, a study of data from 14,778 NHANES participants found significant associations between blood lead and albuminuria (OR 2.34, 95% CI: 1.72, 3.18), reduced eGFR (estimated glomerular filtration rate) (OR 1.98, 95% CI: 1.27, 3.10), and for both outcomes (OR 4.10, 95% CI: 1.58, 10.65) comparing highest to lowest quartiles (Navas-Acien *et al.* 2009). There are also a large number of studies of kidney function among occupational cohorts, as measured by creatinine clearance, serum creatinine or BUN, all clinical indicators of impaired glomerular filtration, or other markers, such as uric acid (Weaver *et al.* 2005), or by early markers of tubular kidney disease, such as excretion of small proteins like β-2-microglobulin or RBP (retinol binding protein), or markers of cytotoxicity, such as NAD (nicotinamide adenine dinucleotide).

A recent comprehensive review (2006) of these studies concluded that lead contributes to nephrotoxicity, even at blood lead levels below 5 µg/dl, especially in people with other illnesses such as hypertension and diabetes (Ekong *et al.* 2006).

Weaknesses in our data include lack of a complete work history to know when exposure began, lack of data on possible confounders, such as hypertension, and possibly inaccurate matching with USRDS and NDI registries, due to the lack of SSN in most of our cohort. Information on SSN was not available for 75% of our cohort, leading to the potential for misclassification of outcome when matching to NDI on name, gender, and date of birth. However, previous work by Williams *et al.* (1992) using subjects known to be dead or alive has shown that with first name, last name, and date of birth investigators can attain a 92% sensitivity (detection by NDI of known dead) and a 92% specificity using NDI (non- matching in NDI for known to be alive) (Williams *et al.* 1992). This work was done having only the first initial of the first name and Williams *et al.* (1992) suggest that specificity will be increased without loss of sensitivity by having the full first name, as we have in ABLES (Williams *et al.* 1992). Use of name and date of birth have also been shown to be effective for NDI by earlier authors (Stampfer *et al.* 1984).

Strengths of our study include a large cohort, documented blood lead levels, and use of ESRD incidence, rather than reliance on chronic renal disease mortality.

CONCLUSION

There is evidence for increased ESRD incidence in our cohort, in both external and internal analysis. Data are limited by the lack of detailed work history and reliance on only a few blood lead tests per person to estimate level of exposure, and lack of information on risk factors, such as hypertension and diabetes.

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Table 5.1 Description of Demographics by Lead category (N=58,307 males).

Category		Highest Lead Category Achieved					
		1	2	3	4	5	Total
		0-<5 µg/dl	5-<25 µg/dl	25-<40 µg/dl	40-<51 µg/dl	51+ µg/dl	
Total		6,832 (11.7%)	18,618 (31.9%)	21,440 (36.8%)	7,348 (12.6%)	4,069 (7.0%)	58,307[*†]
Median Years of Follow-up		6.4	10.2	14.3	17	17.7	12.2
Age at First Test		40.7 (12.2)	40.0 (11.7)	37.9 (11.4)	38.0 (11.4)	38.9 (11.7)	39.0 (11.7)
Race	**White**	1,463 (21%)	2,428 (13%)	6,590 (31%)	2,666 (38%)	1,917 (43%)	15,064 (26%)
	Non-White	258 (4%)	554 (3%)	1,379 (6%)	566 (8%)	430 (105%)	3,187 (5%)
	Missing/Unknown	5,111 (75%)	15,635 (84%)	13,471 (63%)	3,768 (54%)	2071 (47%)	40,056 (69%)
	% non-white among known race	0.15	0.19	0.17	0.18	0.18	0.17
Median number of Observations in those with > 1		2	3	4	6	7	4
% with Single Observations		6108 (89.4%)	12710 (68.3%)	7783 (36.3%)	1445 (19.7%)	494 (12.1%)	28540 (48.9%)
Mean Highest Blood Lead Level		2.5 (1.1)	13.0 (5.5)	30.8 (4.2)	44.7 (3.3)	65.3 (18.7)	25.9 (17.9)
% with SSN (for matching)-Overall[#]		611 (8.9%)	2,079 (11.2%)	7,661 (35.7%)	3,104 (42.2%)	1,778 (43.7%)	15,233 (26.1%)
Median Year of Birth		1962	1961	1959	1956	1954	1959
Median Year of ESRD		2007	2004	2004	2003	2003	2004
Number with ESRD		29 (0.4%)	80 (0.4%)	99 (0.5%)	49 (0.7%)	47 (1.2%)	304 (0.5%)

Table 5.2 Number of people by state and lead category in the cohort (n=58,307)

STATE	Highest Lead Category Achieved					
	1	2	3	4	5	Total
	0-<5 µg/dl	5-<25 µg/dl	25-<40 µg/dl	40-50 µg/dl	51+ µg/dl	
California	1,934 (28%)*	2,663 (14%)	3,916 (18%)	1,294 (18%)	706 (17%)	10,513 (18%)
Connecticut	383 (6%)	581 (3%)	895 (4%)	217 (3%)	126 (3%)	2,202 (4%)
Iowa	373 (5%)	389 (2%)	572 (3%)	242 (3%)	87 (2%)	1,663 (3%)
Massachusetts		2,382 (13%)	2,530 (12%)	830 (11%)	453 (11%)	6,195 (11%)
Michigan	292 (4%)	470 (2%)	790 (4%)	196 (3%)	78 (2%)	1,826 (3%)
Minnesota	190 (3%)	334 (2%)	543 (2%)	153 (2%)	59 (1%)	1,279 (2%)
New Jersey	1,593 (23%)	1,186 (6%)	2,097 (10%)	859 (12%)	553 (14%)	6,288 (11%)
New York		7,681 (41%)	5,491 (26%)	2,357 (32%)	1,429 (35%)	16,958 (29%)
Ohio	1,126 (16%)	1,522 (8%)	2,134 (10%)	719 (10%)	374 (9%)	5,875 (10%)
Pennsylvania	334 (5%)	536 (3%)	1,005 (5%)	123 (2%)	44 (1%)	2,042 (3%)
Wisconsin	607 (9%)	874 (5%)	1,467 (7%)	358 (5%)	160 (4%)	3,466 (6%)

*All percentages are column percentages

Table 5.3 ESRD standardized Incidence Ratios (SIR) and 95% confidence intervals by lead exposure category in the sub-cohort with data on race (n=18,057)*

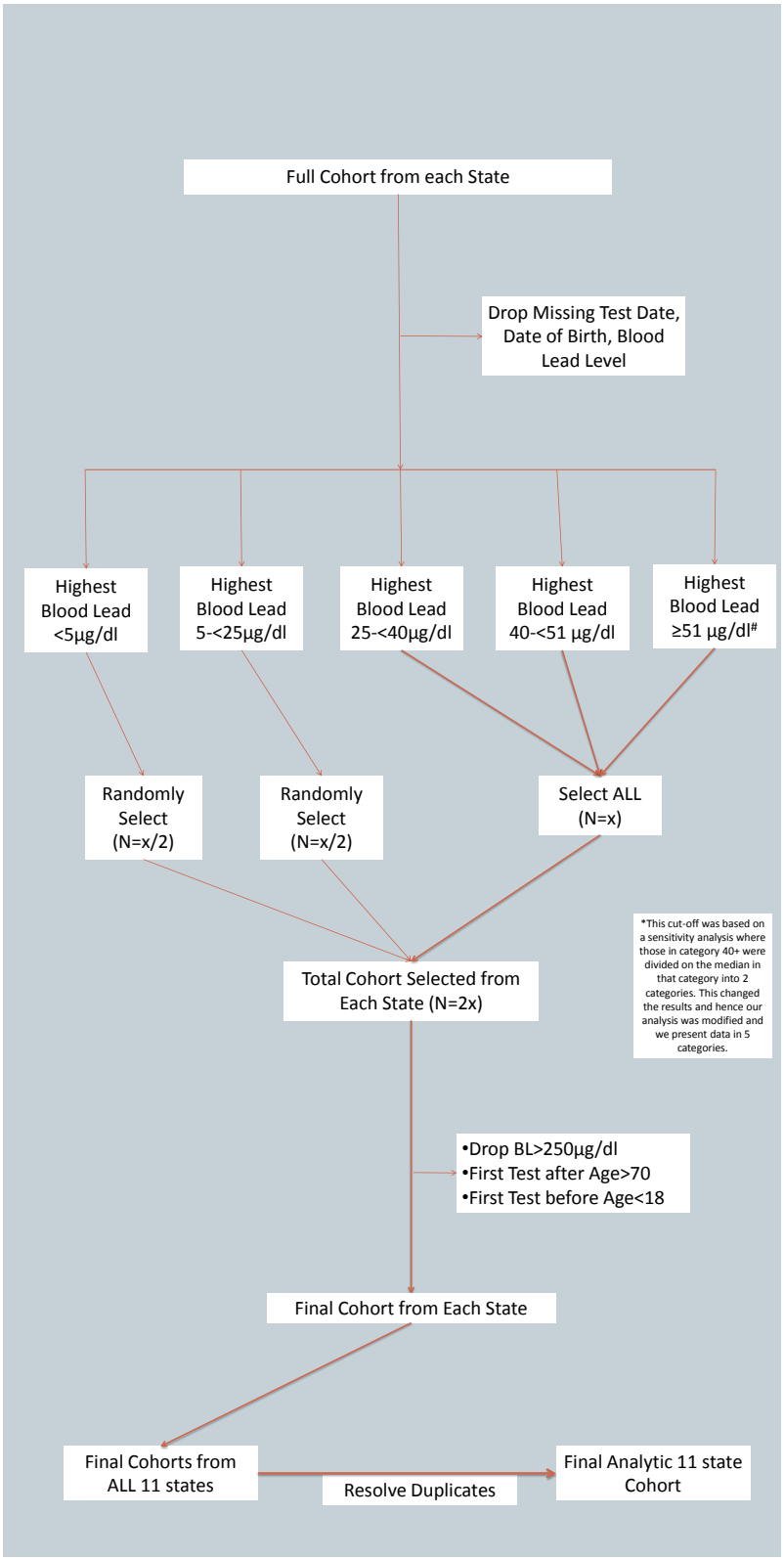
Cause		Highest Lead Category Achieved											
		1		2		3		4		5		Total	
		0-<5 µg/dl		5-<25 µg/dl		25-<40 µg/dl		40-50 µg/dl		51+ µg/dl			
All Causes	Overall	6	1.35 (0.49-2.93)	7	0.69 (0.28-1.42)	41	0.96 (0.69, 1.30)	25	1.10 (0.71, 1.63)	29	1.47 (0.98, 2.11)	108	1.08 (0.89,1.3
	5+ yrs Follow-up	5	2.73 (0.89, 6.36)	3	0.56 (0.11, 1.62)	24	0.74 (0.48, 1.11)	21	1.13 (0.70, 1.73)	26	1.56 (1.02-2.29)	79	1.06 (0.84, 1.3

*males only

Table 5.4 ESRD Standardized Incidence Ratios (SIR) and 95% confidence intervals by blood lead category in full cohort (n=58,307, race imputed for 69%)

Cause		Highest Lead Category Achieved											
		1		2		3		4		5		Overall	
		0-<5 µg/dl		5-<25 µg/dl		25-<40 µg/dl		40-50 µg/dl		51+ µg/dl			
All Causes	Overall	29	1.31 (0.83-1.79)	79	1.00 (0.78, 1.23)	98	0.73 (0.59, 0.88)	44	0.80 (0.56, 1.03)	52	1.36 (0.99, 1.73)	302	0.92 (0.82, 1.03
	5+ yrs follow-up	9	1.03 (0.36, 1.71)	34	0.73 (0.49, 0.98)	65	0.64 (0.49, 0.80)	38	0.85 (0.58, 1.12)	45	1.43 (1.01- 1.85)	191	0.82 (0.71, 0.94
All cause- whites		19	1.72 (1.03-2.68)	52	1.13 (0.84-1.48)	65	0.90 (0.69-1.14)	26	0.86 (0.56-1.26)	27	1.22 (0.80-1.78)	189	1.04 (0.90-1.20
All causes – non-whites		10	0.90 (0.43-1.87)	27	0.85 (0.56-1.24)	33	0.54 (0.37-0.76)	18	0.72 (0.42-1.13)	25	1.55 (1.01-2.29)	113	0.78 (0.64-0.93
Diabetes		13	1.25 (0.67-2.14)	27	0.74 (0.49-1.07)	36	0.59 (0.41-0.82)	13	0.52 (0.28-0.89)	21	1.22 (0.75-1.86)	110	0.73 (0.60-0.88
Hypertension+ Glomerular disease		9	1.16 (0.77-1.55)	36	1.36 (1.14-1.59)	34	0.75 (0.62-0.88)	14	0.71 (0.52-0.89)	17	1.25 (0.94-1.55)	110	0.96 (0.86-1.05

Figure 5.1 Cohort Selection Flowchart



CHAPTER 6. Survival patterns in a cohort of lead exposed workers with End Stage Renal Disease (ESRD) from the Adult Blood Lead Epidemiology & Surveillance (ABLES) program.

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Running Title: Survival patterns in a cohort of lead exposed workers with End Stage Renal Disease (ESRD) from the Adult Blood Lead Epidemiology & Surveillance (ABLES) program.

Key Words: Mortality, Lead, Cancer, Stroke, Heart Disease, Hypertension, Kidney disease, Occupational Exposure, ABLES, ESRD

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Abbreviations: ABLES: Adult Blood Lead Epidemiology & Surveillance

BL: Blood Lead

ESRD: End Stage Renal Disease

ABSTRACT

Background: Current evidence indicates that lead exposure increases blood pressure in adults, and may contribute to chronic renal disease. We hypothesized that blood lead level might be associated with survival after diagnosis with End Stage Renal Disease (ESRD).

Methods: The population was formed of subjects with at least one blood lead test that were part of a NIOSH-sponsored occupational lead surveillance program (ABLES) in 11 states and were diagnosed with ESRD. The survival of people in various categories of lead exposure was studied after adjusting for potential confounders. Blood lead (BL) categories were defined by highest BL test, and were 0-<5 µg/dl, 5-<25 µg/dl, 25-<40 µg/dl, 40-<50 µg/dl and 50+ µg/dl. Cox proportional hazards models were run to test the hypothesis.

Results: There were 434 ESRD cases with 82% males, 65% White and 31% African American. Fifty-one percent had only one blood test, which the remainder had a median of five tests. The median years of follow-up in the cohort were 2.7 years and there were 219 deaths in the cohort. After adjusting for covariates (e.g. transplantation status, age at diagnosis, glomerular filtration rate, comorbidities, and ethnicity), we found no association between highest measured blood lead level and mortality across categories; 0-<5 µg µg/dl (HR1.00), 5-<25 µg/dl (HR=1.09, 95% CI 0.70, 1.70), 25-<40 µg/dl (HR=1.28, 95% CI 0.81, 2.02), 40-<50 µg/dl (HR=0.89, 95% CI 0.48, 1.63) and 50+ µg/dl (HR=1.09, 95% CI 0.66, 1.81).

Conclusion: We found no association between blood lead level and survival after ESRD diagnosis.

INTRODUCTION

Lead is neurotoxic in children (Bryce-Smith 1972, Blackwood 1975, de la Burde *et al.* 1975, Valdes Bolanos 1975), and can cause acute poisoning in adults (White 1975, Agency for Toxic Substances and Disease Registry 2007, Garcia-Leston *et al.* 2010). Adult chronic lead exposure has been associated with kidney dysfunction, and with increased incidence of, and mortality from non-malignant kidney disease. However, the evidence is not conclusive.

With the EPA's establishment of permissible level of lead in the air (U.S. Environmental Protection Agency 1977), and subsequent reduction of lead use in commercially available products (particularly leaded gasoline), ambient lead exposure has been greatly reduced. Nonetheless, there continues to be substantial occupational exposure to lead. The National Institute of Occupational Safety and Health (NIOSH) estimates more than 3 million workers in the US were potentially exposed to lead at work in the 1980s (Rempel 1989, Staudinger *et al.* 1998).

13% of US adults have diagnosed chronic kidney disease, with increasing numbers each year (Coresh *et al.* 2007). Very high levels of lead in the body are known to result in kidney failure(U.S. Environmental Protection Agency 2005), but whether lower levels can result in chronic renal disease is not clear, although the data are suggestive. Multiple studies have demonstrated the worsening of kidney function (serum creatinine, decreased creatinine clearance) among those exposed to lead, even at low doses of lead exposure (Chia *et al.* 1995, Lin *et al.* 2001, Lin *et al.* 2003, Weaver *et al.* 2003, Tsaih *et al.* 2004, Lin *et al.* 2006, Weaver *et al.* 2009). A recent (2006) comprehensive review of lead-related nephrotoxicity (Ekong *et al.*

2006) concluded that lead contributes to nephrotoxicity, even at blood lead levels below 5 µg/dl, especially in people with other illnesses, such as hypertension and diabetes. Several occupational studies have shown excess chronic kidney disease (CKD) with lead exposure. (Fanning 1988, Steenland *et al.* 1992), but these results are based on small numbers and are not conclusive. Lead exposure association with chronic kidney disease (CKD), at common occupational levels, remains inconclusive.

However, to date, no study has considered blood lead as a risk factor associated with survival/mortality among incident ESRD cases developing after exposure to lead. Although there are studies among the general population looking at survival after diagnosis of ESRD, none have considered blood lead as a risk factor either (Kucukkoylu *et al.* 2013, Tangri *et al.* 2013).

In prior work, we found suggestive evidence that lead exposure may be associated with end-stage renal disease (ESRD) (Chowdhury *et al.* 2013) and mortality (Chowdhury *e al.* 2013a). Our present study aims to study the effect of lead exposure in categories with survival among occupationally exposed lead workers who have developed ESRD, adjusting for other variables. In particular, we were interested if blood lead, as measured before ESRD developed, was associated with worse survival after ESRD.

METHODS

Data Sources/Study Participants

The Adult Blood Lead Surveillance (ABLES) program, sponsored by the National Institute for Occupational Safety and Health (NIOSH), started collecting state-level data on blood lead exposure since 1987 (Roscoe *et al.* 2002). In participating states, state agencies collected data on all subjects tested in any laboratory in the state doing blood lead tests.

NIOSH has collected data on industry for a limited number of ABLES subjects (n=6,999) (National Institute for Occupational Safety and Health 2012). Of these, 62% were in manufacturing, 10% in construction, 7% in metal mining, 1% in trade (scrap and waste materials), and 20% were in other industries or data were unavailable.

We obtained data from 11 state ABLES programs: Connecticut, California, Ohio, Minnesota, Iowa, Pennsylvania, New York, New Jersey, Wisconsin, Michigan and Massachusetts, from their year of first participation through end 2008. We excluded everyone who was tested after 2005, to avoid very short follow-up time. We also excluded any subject missing information on date of birth, test date, or blood lead levels and observations with blood lead level greater than 250µg/dl, as these values were considered implausible. We further excluded all people who were tested for the first time after the age of 70 years or before the age of 18 years, as these were more likely to be acute exposures, and hence unlikely to be occupational exposures. We also wished to analyze a possibly occupationally-exposed cohort. Before we had applied the last criteria, a dataset had been sent to USRDS with data from 6 states: California, Connecticut, New Jersey, Iowa, Ohio and Michigan. After application of this criterion to all 5 categories and random selection from categories 1 and 2, we noticed that there was a subset of approximately 12,000 people who were not a part of our new cohort. Though these would have been excluded, USRDS had already provided us with 100 ESRD matches/cases in this sub group. We decided to include them in our further analysis for this paper. We also sent this group to NDI for matching for death information. We then further searched for duplicate records across states using combinations of SSN, last name and date of birth and found 2300 duplicate records with tests in different states. These were assigned the same identity number

across states for uniformity; and those duplicates which we were unable to distinguish were dropped from the analysis.

We categorized each blood lead test into 1 of 5 categories, namely <5 µg/dl, 5 to <25 µg/dl, 25 to <40 µg/dl, 40 to <50 µg/dl, and 50+ µg/dl or categories 1 through 5, respectively. Categories <25, 25-40, and 40+ have been traditionally used to categorize occupational blood leads, while the lowest category 5 µg/dl essentially was equivalent to non-occupational US blood lead levels. We subdivided the highest category at 50 µg/dl, which is the OSHA cutoff for removing subjects from lead exposure until their blood lead level drops below 40 µg/dl. We then assigned a final single blood lead category for each subject, defined as the highest category ever achieved by an individual.

We first selected everyone from the states who had ever had a blood lead level reading in categories 3 or 4 or 5. We then selected an equal number of people from categories 1 and 2 (50% from each category), stratified by state. We the matched this cohort against the National Death Index (NDI) to obtain vital status information and the US Renal Data System (USRDS) to determine who had developed incident ESRD after having been previously tested for blood lead. The last three states (Wisconsin Michigan and Massachusetts) opted to do their own data processing and matching with the National Death Index (NDI) and United States Renal Data System (USRDS). They, too, followed the same selection pattern but independently submitted data to NDI and USRDS and sent us de-identified data.

We used name, date of birth, gender, race (when available) and SSN (when available) for matching with the NDI and USRDS databases till end of 2010. Similar matching of other occupational cohorts with USRDS for renal disease incidence has been done in the past (Calvert *et al.* 1997, Steenland *et al.* 2001, Radican *et al.* 2006).

Follow-up of our cohort for renal disease incidence, via matching with the USRDS, and for vital status via NDI, was through 2010. For the three states which did their own matching and sent us de-identified data s (~15% of the cohort, Wisconsin, Massachusetts and Michigan), follow-up went through 2009.

To determine if a match with the NDI was a true match from amongst the multiple matches reported by NDI, we only selected those who were assigned a status code of 1 by NDI, indicating a high probability of a match. If person's last blood lead date was after their date of death, then the match was false, and we dropped all information received from NDI, i.e. these subjects were considered alive. If there were multiple matches with status code 1, we selected the one the NDI reported as an exact match. If there was no exact match, we sorted all the status codes=1 by probability score. If highest probability score was ≥ 40 and state of death was the same as the state where a subject was tested, then we selected that observation. If there were multiple matches meeting this criterion, then we selected the one with the higher probability score of match. If we are unable to select a match based on the above criteria, we dropped those observations entirely from the final dataset to avoid misclassification of outcome.

With regard to USRDS, anyone who USRDS considered a match was accepted and was considered to be an ESRD case. Of these cases, 137 had been diagnosed with ESRD prior to their first blood lead test date and were excluded, and hence we were left with 434 unique ESRD cases for the present study. We also obtained data on date of death and cause of death for ESRD cases from the USRDS as the USRDS follows all ESRD cases longitudinally. If an ESRD case was not declared dead by NDI, but had been reported as dead by the USRDS, we considered the person to be dead. The USRDS uses the Social Security Administration (SSA) to determine deaths. In addition to standard list of matching variables, we also requested the USRDS to

provide detailed information on the following Core Standard Analysis Files (SAF) data-Treatment History (RXHIST), Medical Evidence (MEDEVID95), Medical Evidence (MEDEVID05), Death information (DEATH), Transplant (TX) and patient information (Patients). These data sets provided us with information on glomerular filtration rate (GFR) at time of ESRD diagnosis, body mass index (BMI), race, co-morbidity, transplant status, type of medical insurance, and Spanish ethnicity. All of which we considered potential confounders of a possible association between lead exposure and mortality.

We used Cox Proportional Hazards models to evaluate association of survival pattern and lead exposure level the in five aforementioned categories among ESRD cases, after adjusting for covariates including age at first test, race, ever transplanted, glomerular filtration rate (GFR) before start of dialysis, body mass index (BMI), year of ESRD diagnosis (for cohort effect) and co-morbidities -specifically chronic obstructive pulmonary disease (COPD) and any cardiac disease. Other variables were not included in the model, as they were not associated with mortality at the p=0.10 level in univariate analyses.

GFR and age at ESRD diagnosis were modeled as continuous variables, as they showed a monotonic trend when examined in quartiles and quintiles, respectively. BMI was divided into 4 categories – underweight (<18.5 kg/m^2), normal (18.5-<24.9 kg/m^2), overweight (24.9-<30 kg/m^2) and obese ($\geq$30kg/m^2).

We used backwards elimination to reduce our full model to final models using Akaike Information Criteria (AIC) and p-values (variables with p-values >0.1 were dropped). Lead exposure, our key variable of *a priori* interest, was retained in all models.

We examined interaction terms between lead exposure and the following variables-transplantation status, age at ESRD diagnosis, GFR and race, which were the only variables

which retained in our backwards elimination. None of the interaction terms were significant at alpha=0.05, and hence all interaction terms were dropped. The model without interaction terms also had a lower AIC than models with interaction terms in them. Next, we assessed confounding.

We further assessed the proportional hazards assumption for lead exposure via interactions of lead exposure category with time (follow-up time between ESRD diagnosis and end of follow-up) for our final model. Proportional hazards assumption for lead was not violated in the final model.

SAS version 9.3 (SAS Institute, Cary, NC) was used for all analysis.

RESULTS

Table 6.1 provides descriptive information about the cohort. Of 434 ESRD cases, there were 82% males; 65% were White and 31% African American. Fifty-one percent had only one blood test, while those with multiple tests had a median of 5 tests. The median years of follow-up in the cohort were 2.7 years, and there were 219 deaths in the cohort. In general, people in the highest category, compared to the lowest category, tended to have higher median number of blood lead tests, more complete information on SSN, fewer transplants, earlier birth year, onset of ESRD and death.

Table 6.2 displays the univariate results of the Cox PH models for lead and other variables in relation to mortality. In univariate models, the data show some suggestion of an increase in mortality risk with greater blood lead, especially for the highest blood lead category. GFR in quartiles and age at ESRD diagnosis in quintiles show a monotonic increase in hazard of mortality, and hence we subsequently modeled them as continuous variables.

Table 6.3 displays the final fully adjusted model after excluding variables with p>0.1. There is no longer any suggestion of a positive trend in mortality risk with increased blood lead category. A highly significant association with mortality is seen for non-transplanted patients (for non-transplanted HR=7.46, 95% CI 3.72, 14.97). Both GFR (HR=1.06, 95% CI 1.03, 1.10), and age at ESRD diagnosis (HR=1.03, 95% CI 1.01, 1.05) show strong trends of increased mortality risk for each unit of GFR and each year of age increase, respectively.

DISCUSSION

Lead exposure by category was not a significant predictor in multivariate models, and nor did it show a monotonic increasing trend with increasing category of lead exposure. Our study cohort, from whom the ESRD cases were derived, was relatively young. It is possible that a blood lead effect of survival might emerge as the cohort ages, with more ESRD cases and longer follow-up time.

We found no increase in mortality with higher blood lead level among ESRD patients. . Our null finding could be biased, however. Risk factors associated with increased risk of both death and certain chronic diseases in general populations, have been found previously to be associated with paradoxically lower mortality among those with the disease (Kokkinos *et al.* , Oreopoulos *et al.* 2008, Lavie *et al.* 2009, Carthenon *et al.* 2012, Bucholz *et al.* in press). More specifically, among ESRD patients, such findings have been seen with creatinine, systolic and diastolic blood pressure (BP) and high cholesterol levels (Kopple 2005, Kovesdy *et al.* 2007). Recently, obesity was found to show a similar paradoxical association, wherein, in spite of evidence indicating an association of obesity with both ESRD and premature death in general

populations (Lew *et al.* 1979, De Gonzalez 2011, Kalaitzidis *et al.* 2011), obese ESRD patients were found to live longer on average as compared to non-obese ESRD patients (Kopple 2005). Several explanations have been put forth to explain these paradoxical observations in these studies, including bias due to selection of a diseased population, model misspecification, presence of unmeasured confounding (Dahabreh *et al.* 2011) and competing risk (personally communication with William McClellan and Dana Flanders 2013). In the case of obesity, others have even posited that the effect of obesity on mortality may differ between ESRD and non-ESRD populations (Kalantar-Zadeh 2007, Levin *et al.* 2007). Flanders *et al*.(2013) (Flanders *et al.*2013) have also tried to explain this paradox by providing a possible mechanism in which differential, harmful effects of obesity on both ESRD occurrence and death can lead to lower mortality among obese rather than non-obese subjects after ESRD onset.

Our study has a number of limitations. We did not have any information on smoking and alcohol consumption. Smoking and alcohol consumption are strong risk factors for mortality, and may be associated with lead levels. Another important limitation is that we do not have work history, and we have limited data on blood lead levels over time. While we are reasonably confident that most of those in our cohort were exposed occupationally, we do not know when lead exposure began, so that analyses by latency are not possible. Another limitation is the lack of SSNs on 75% of our cohort, and the potential for misclassification of outcome when matching to NDI on name, gender, and date of birth. However, previous work by Williams *et al*. (1992) (Williams *et al.* 1992), using subjects known to be dead or alive, has shown that with first name, last name, and date of birth investigators can attain a 92% sensitivity (detection by NDI of known dead) and a 92% specificity using NDI (non- matching in NDI for known to be alive).

This work was done having only the first initial of the first name, and Williams *et al*. (1992) (Williams *et al.* 1992) suggest that specificity will be increased without loss of sensitivity by having the full first name, as we have in ABLES. Use of name and date of birth have also been shown to be effective for matching with NDI database by earlier authors (Stampfer *et al.* 1984). While we do not have analogous information on the accuracy of matching to the USRDS without SSN, we believe the accuracy is likely to be similar to that of NDI. Similarly, data received from USRDS was considered to be a unique match with high degree of accuracy and precision as per conversations with Beth Forrest, our liaison officer at USRDS. Validation studies have shown that about 95% of all US patients with ESRD are in the USRDS system (1992, USRDS 1992).

It should also be noted that our study has a number of strengths, the most important of which are a large study population with documented blood lead levels from which this data were derived. The strongest predictor of survival was transplantation status with those never transplanted having a HR of 8.11(4.15, 15.86). Other significant predictors of mortality were GFR (HR=1.07, 95% CI 1.03, 1.10) and age at ESRD diagnosis (HR=1.03, 95% CI 1.01, 1.04). This provides further support to existing literature on the association of transplantation and survival (Wong *et al.* 2012, Rocha *et al.* 2013), increased GFR and worse outcomes (Susantitaphong *et al.* 2012) and age at ESRD diagnosis and survival.

CONCLUSION

There is no evidence of increased risk of mortality among ESRD cases with higher lead exposure compared to those with low lead exposure. However, the strongest associations for increased risk of mortality were higher GFR at diagnosis, not being transplanted, and later age at onset of ESRD, which conform to findings in literature. Limitations are lack of work history, and

absence of data on smoking. Since this is a relatively young cohort, re-examination of these associations after a few years may shed more light on the survival patterns among lead exposed workers with ESRD.

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Table 6.1 Demographics of the Cohort

Category		Highest Lead Category Achieved					
		1	2	3	4	5	Total
		0-<5 µg/dl	5-<25 µg/dl	25-<40 µg/dl	40-<50 µg/dl	50+ µg/dl	
Total		92 (21.2%)	120 (27.7%)	113 (26.0%)	46 (10.6%)	63 (14.5%)	434
Age at First Test‡		49.7 (13.7)	50.6 (12.3)	45.4 (12.4)	44.1 (11.2)	46.8 (12.2)	47.8 (12.7)
Male		54 (58.7%)	99 (82.5%)	103 (91.2%)	43 (93.5%)	57 (90.5%)	356 (82.0%)
Race	**White**	64 (69.6%)	81 (67.5%)	75 (66.4%)	25 (54.4%)	37 (58.7%)	282 (65.0%)
	Black	21 (22.8%)	32 (26.7%)	35 (31.0%)	21 (45.6%)	24 (38.1%)	133 (30.7%)
	Other	7 (7.6%)	7 (5.8%)	3 (2.6%)	0 (0.0%)	2 (58.7%)	19 (4.3%)
Median number of Observations in those with > 1 BL Test*		2	2	4	8	9	5
% with Single Observations		85 (92.4%)	86 (71.7%)	35 (31.0%)	10 (21.7%)	4 (6.3%)	220 (50.7%)
Median Highest Blood Lead Level		2	10	30	43	60	25
Mean Highest Blood Lead Level‡		2.4 (1.1)	11.0 (5.2)	30.8 (4.3)	43.6 (2.7)	65.5 (18.0)	25.7 (22.5)
% with SSN (for matching)-Overall#		11 (12%)	13 (10.8%)	40 (35.4%)	30 (65.2%)	39 (61.9%)	133 (30.7%)
Median Year of Birth		1952	1946.5	1948	1946.5	1943	1947
Median Year of ESRD		2006	2005	2004	2005	2003	2005
Median Year of Death		2007	2006	2006	2005	2004	2006
Number of Deaths		32 (34.8%)	61 (50.8%)	61 (54.0%)	24 (52.2%)	41 (65.1%)	219 (50.5%)
Median Years of Follow-up		2.4	2.9	2.7	3.6	2.9	2.7
Comorbidities$		53 (58.2%)	76 (63.9%)	56 (53.3%)	21 (51.2%)	34 (60.7%)	240 (58.3%)
Ever Transplanted		18 (19.6%)	29 (24.2%)	26 (23.0%)	10 (21.7%)	10 (15.9%)	93 (21.4%)
Median GFR		9.6	9.4	7.9	8.7	9.5	9.1
Median BMI		27.1	27.5	27.7	27.1	27	27.4
Hispanic	**Yes**	21 (22.8%)	17 (14.2%)	15 (13.3%)	5 (10.9%)	11 (17.5%)	69 (15.9%)
	No	70 (76.1%)	102 (85.0%)	90 (79.7%)	34 (73.9%)	45 (71.4%)	341 (78.6%)
	Unknown	1 (1.1%)	1 (0.8%)	8 (7.0%)	7 (15.2%)	7 (11.1%)	24 (5.5%)

Insurance Type	**None**	32 (34.8%)	31 (25.8%)	27 (23.9%)	9 (19.6%)	12 (19.1%)	111 (25.6%)
	Group/Other	59 (64.1%)	88 (73.3%)	78 (69.0%)	32 (69.6%)	44 (69.8%)	301 (69.4%)
	Missing/Unknown	1 (1.1%)	1 (0.8%)	8 (7.1%)	5 (10.8%)	7 (11.1%)	22 (5.1%)

Some states like WI, MI, MA and PA sent their own data and sent us de-identified data so these may be underestimates

*There were people missing observations in the dataset- GFR (n=30), BMI (n=7), and comorbidities (n=22)

[$]Comorbidities included were Chronic Obstructive Pulmonary Disease and Any Cardiac comorbidity. Data on other comorbidities were incomplete to a greater extent or absent.

‡ Mean and standard deviation

Table 6.2 Univariate Hazard Ratios and 95% Confidence Intervals. (n=434)

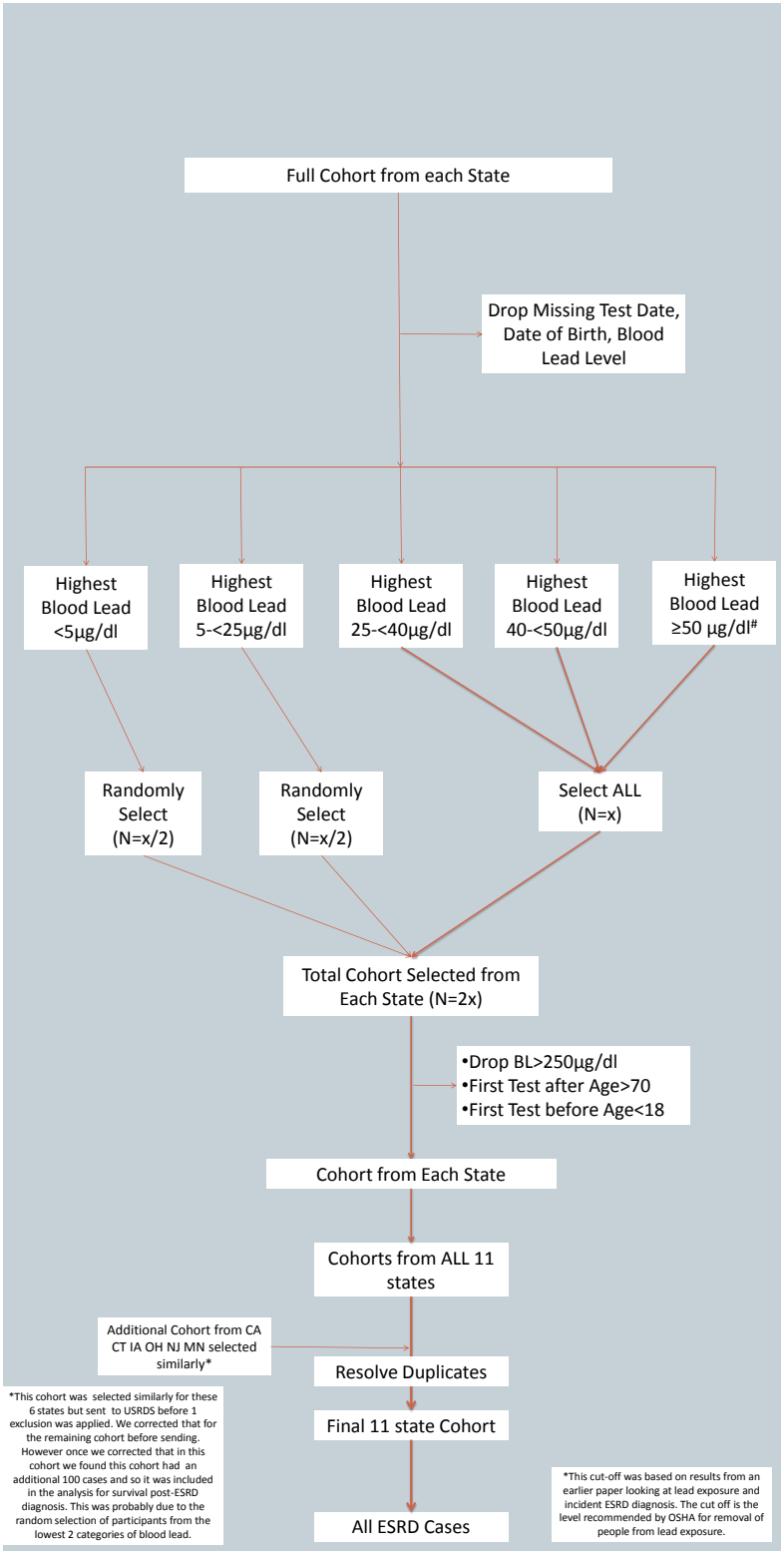
Predictor			Univariate Models
Lead Category	**1**	**0-<5 µg/dl**	REF
	2	**5-<25 µg/dl**	1.22 (0.79, 1.88)
	3	**25-<40 µg/dl**	1.23 (0.79, 1.90)
	4	**40-<50 µg/dl**	1.12 (0.65, 1.92)
	5	**50+µg/dl**	1.51 (0.94, 2.41)
Ever Transplant	**No**		7.85 (4.76, 12.94)
BMI		**kg/m^2**	0.98 (0.96, 1.00)
BMI Categories	**Underweight**	**<18.5 kg/m^2**	REF
	Normal	**18.5-<24.9 kg/m^2**	0.63 (0.34, 1.17)
	Overweight	**24.9-<29.9 kg/m^2**	0.55 (0.30, 1.02)
	Obese	**≥29.9 kg/m^2**	0.46 (0.25, 0.87)
GFR		**ml/min/1.73m^2**	1.08 (1.05, 1.11)
GFR in Quartiles	**Quartile 1**	**0-<6.5 ml/min/1.73m^2**	0.39 (0.26, 0.59)
	Quartile 2	**6.5-<9.1 ml/min/1.73m^2**	0.66 (0.44, 0.97)
	Quartile 3	**9.1-<12.4 ml/min/1.73m^2**	0.77 (0.52, 1.13)
	Quartile 4	**≥12.4 ml/min/1.73m^2**	REF
Age At ESRD Diagnosis		**Years**	1.05 (1.04, 10.6)
Age At ESRD Diagnosis in Quintiles	**Quintile 1**	**0-<44.2 years**	REF
	Quintile 2	**44.2-<52.9 years**	1.37 (0.81, 2.31)
	Quintile 3	**52.9-<60.9 years**	2.49 (1.54, 4.05)
	Quintile 4	**60.9-<67.7 years**	4.18 (2.59, 6.73)
	Quintile 5	**≥67.7 years**	5.23 (3.26, 8.38)

Table 6.3 Hazard Ratios and 95% Confidence Intervals for full and final reduced models (n=434)

Predictor		Final Model	p-value for Test of Trend
Lead Category	**0-<5 µg/dl**	REF	0.93
	5-<25 µg/dl	1.09 (0.70, 1.70)	
	25-<40 µg/dl	1.28 (0.81, 2.02)	
	40-<50 µg/dl	0.89 (0.48, 1.63)	
	50+µg/dl	1.09 (0.66, 1.81)	
Ever Transplant	**No**	8.11 (4.15, 15.86)	
GFR‡		1.07 (1.03, 1.10)	
Age At ESRD Diagnosis‡		1.03 (1.01, 1.04)	
Ethnicity	**Non-Hispanic**	REF	
	Hispanic	0.65 (0.42, 1.02)	

‡ Hazard Ratios are for 1 year increase in Age at ESRD diagnosis and 1 ml/min/1.73m^2 increase in GFR

Figure 6.1 Cohort Selection Flowchart


Full Cohort from each State
Drop Missing Test Date, Date of Birth, Blood Lead Level
Highest Blood Lead <5µg/dl
Highest Blood Lead 5-<25µg/dl
Highest Blood Lead 25-<40µg/dl
Highest Blood Lead 40-<50µg/dl
Highest Blood Lead ≥50 µg/dl#
Randomly Select (N=x/2)
Randomly Select (N=x/2)
Select ALL (N=x)
Total Cohort Selected from Each State (N=2x)
•Drop BL>250µg/dl
•First Test after Age>70
•First Test before Age<18
Cohort from Each State
Cohorts from ALL 11 states
Additional Cohort from CA CT IA OH NJ MN selected similarly*
Resolve Duplicates
Final 11 state Cohort
All ESRD Cases
*This cohort was selected similarly for these 6 states but sent to USRDS before 1 exclusion was applied. We corrected that for the remaining cohort before sending. However once we corrected that in this cohort we found this cohort had an additional 100 cases and so it was included in the analysis for survival post-ESRD diagnosis. This was probably due to the random selection of participants from the lowest 2 categories of blood lead.
*This cut-off was based on results from an earlier paper looking at lead exposure and incident ESRD diagnosis. The cut off is the level recommended by OSHA for removal of people from lead exposure.

CHAPTER 7. CONCLUSION

Lead Exposure and Mortality

The strongest associations between lead exposure by category and cancer were for lung cancer in both external and internal analyses, with a modest excess in the highest blood lead category. There was also some suggestion of an increase of larynx cancer in the highest blood lead level group, but based on small number of deaths. Our finding of excess larynx cancer with lead exposure is new among existing studies. No other cancers of *a priori* interest were associated with blood lead level, although the number of deaths was small for many of them. Overall, for cancer outcomes, our results provide further support to the thesis that there is a causative association of lead exposure in subsequent development of lung cancer. We did not find associations with stomach, kidney and brain cancer, other outcomes of *a priori* interest. This is possibly due to the relatively young nature of the cohort, and the small number of deaths from these causes.

For cardiovascular outcomes, we found a decreased risk of CVD mortality in external comparison, likely as a result of healthy worker effect (HWE) which particularly affects cardiovascular disease. In internal comparisons, we found a statistically significant positive trend in heart disease mortality with increased BL category (p-trend<0.0001). However, this result must be interpreted with caution given the different lengths of follow-up period by BL categories which would affect internal comparisons to a greater extent. Further follow-up of our cohort will help clarify the heart disease findings. Despite small numbers, in internal comparisons, we found a significant

monotonic increase in mortality due to non-malignant kidney disease (p-trend=0.04) but there were no significant associations between any category of lead exposure and mortality.

Lead Exposure and Incident End Stage Renal Disease (ESRD)

We found evidence of an association of lead exposure and increased ESRD incidence, both in external comparison and internal comparison, for those in the highest blood lead category (≥51 µg/dl). We found this evidence both in analyses restricted to the 31% of the cohort with known race information available from the states where our data originated, as well as in the entire cohort for which we imputed race for whom it was missing Furthermore, in the full cohort, we found a strong positive trend of increased risk with increased BL category (p=0.003). It is possible that our finding of an excess of ESRD in the highest blood category reflects the wearing off of the healthy worker effect (HWE) in this largely occupational cohort, which showed some evidence overall of a HWE (SIR overall of 0.92 in the entire cohort). However we found an excess for this category in the sub-analysis for known rate which did not show a HWE (SMR 1.08). The excess we found in the highest BL category was particularly pronounced among those with more than 5 years follow-up from first blood lead test. This conforms to the idea that findings for those with short follow-up may not be the result of a chronic disease process; although given our lack of detailed work history we do not know the true data of initial lead exposure. For those with short follow-up, it is also possible that blood lead testing

did not result from occupational surveillance but from clinicians testing for lead when faced with patients with incipient renal disease, although we have no data on this point. The excess in the highest category was more pronounced for non-whites than whites. Non-whites are known to have much higher ESRD rates than whites, presumably due in part to the higher prevalence of hypertension in this group, which is primarily composed of African-Americans. Our finding may imply that high lead exposure exacerbates the already high underlying risk for the non-white group. It should be noted that the highest BL group in our study (7% of the cohort) was composed of those with blood leads of 51µg/dl or more, a level (≥50 µg/dl) for which OSHA requires removal of a worker to a lower-exposed job until his/her BL is lowered below 40 µg/dl. Our data re-inforce the need for enforcement of this OSHA standard.

Lead, ESRD and Survival

Lead exposure by category was not a significant predictor in multivariate models, nor did it show a monotonic increasing trend with increasing category of lead exposure. Our study cohort from whom the present study was derived was relatively young and a clearer pattern of association might develop as the cohort ages. Thus, although lead exposure was found to be a significant predictor of mortality due to lung cancer, laryngeal cancer, and ischaemic heart disease and was a significant predictor of ESRD incidence, it was not associated with patient survival after development of ESRD.

Strengths

Our study has many strengths, including a large sample size that was much larger than any previous study examining the impacts of lead exposure. We also had documented blood lead levels, and the ability to conduct internal comparisons for high vs. low dose. For example, the cohort of those with BLLs≥25 μg/dl comprised a cohort larger than any other occupational cohort studied in the past. This presented a unique opportunity to resolve outstanding questions about the health effects of lead exposure. In particular, while previous studies on lead exposure have only assessed impacts on kidney disease mortality, our current study examined this as well as incident ESRD. Further we evaluated the association of lead exposure with survival after diagnosis with ESRD adjusting for potential confounders, which to our knowledge has not previously been considered.

Limitations

Limitations of the current study were the lack of detailed work history and limited ability to conduct continuous exposure-response analyses (i.e., we were limited to categorical data for exposure based on single BLL measurements). While we are reasonably confident that most of those in our cohort were exposed occupationally, we do not know when lead exposure began, so that analyses by latency are not possible. Although we have limited data on blood lead levels over time, a number of previous studies have also detected significant positive dose-response trends using single blood

lead measurements, and our analyses indicated that single measurements for those subjects with multiple measurements were generally representative of all measurements (vide supra).

A potential limitation is the use of categories for lead exposure instead of a continuous BLL measure. The reason for such categorization is the inherent uncertainty in blood lead measurement, i.e., how different is a measurement of 28µg/dl from a measure of 31µg/dl? Since a BLL measure is not reflective of the exact and complete exposure to lead (as lead in the body is not just distributed in the blood but also stored in other tissues like bone), taking only the BLL into account ignores the lead distributed in other compartments in the body. Hence, categorization of the continuous BLL overcomes this problem to some extent and provides a more accurate measure of the actual total lead exposure. Since lead is absorbed and excreted in the duodenum, a person would lose very little lead over time. With more than 95% of the excreted lead being reabsorbed, a person was unlikely to change categories and thus having a single blood lead measure could be considered to be stable measure. Thus, categorization would reflect a better measure over time as opposed to a continuous measure, especially among those with only one BLL measurement.

Another potential limitation is our use of blood lead levels instead of bone lead, a measure of the lead content in bones that can be measured from different bones, commonly the tibia or patella. Other studies have looked at health outcomes and tried to relate them to lead exposure. Since bone lead levels are dependent on the activity levels and bone remodeling/resorption (during healing of fractures, growth spurts, pregnancy,

post-menopause (Webber *et al.* 1995, Korrick *et al.* 2002), hyperthyroidism (Goldman *et al.* 1994), etc., that takes place, bone lead levels may vary with the bone being used to measure lead levels. However, blood lead levels are a measure of circulating lead that indicates acute changes in external and internal (mobilization from tissues) lead exposure, while bone lead reflects long-term exposure. Thus, for chronic disease development bone lead would presumably be most relevant. Measured together, these would form a valuable estimate of total lead exposure in the body. However, bone lead is both expensive and difficult to measure. Consequently many studies measure only blood lead. Multiple blood lead measures integrated over time form an acceptable alternative for cumulative lead exposure and does not need bone lead measurement as they are found to be well-correlated with bone lead (Somervaille *et al.* 1988, Roels *et al.* 1995). In the Nurses' Health Study (NHS), Korrick *et al*. (2002) (Korrick *et al.* 2002), using a sample of 264 Bostonian women, found that blood lead levels track very well independently with both patella and tibial lead levels. Thus the use of blood lead levels can be justified in studies associating lead levels to chronic diseases. However, another study of approximately 1,000 subjects, 50–70 years of age had a mean blood lead level of 4 μg/dl and mean tibia lead level of 19 μg/g, with a Pearson's r correlation of only 0.12 (Schafer *et al.* 2005, Martin *et al.* 2006). Despite these conflicting data on the correlation of bone and blood lead, BLL is commonly used instead of bone lead in epidemiologic studies because bone lead measurements are invasive, expensive, and time consuming, and generally not feasible for large cohort studies.

We did not have any information on smoking and alcohol consumption. Smoking and alcohol consumption are strong risk factors for mortality, and may be associated with lead levels. Smoking is also a potential confounder for our principal findings of interest, lung cancer and larynx cancer. However, apart from lung and larynx cancer, most other smoking-related outcomes show no excess in the highest blood lead category (i.e., bladder cancer, heart disease, COPD, esophageal cancer).

Another limitation is the lack of SSNs for 75% of our cohort, and the potential for misclassification of outcome when matching to NDI on name, gender, and date of birth. However, previous work by Williams *et al*. (1992) (Williams *et al.* 1992) using subjects known to be dead or alive has shown that with first name, last name, and date of birth investigators can attain a 92% sensitivity (detection by NDI of known dead) and a 92% specificity using NDI (non- matching in NDI for known to be alive). This work was done having only the first initial of the first name, and Williams *et al*. (1992) (Williams *et al.* 1992) suggest that specificity will be increased without loss of sensitivity by having the full first name, as we have in ABLES. Use of name and date of birth have also been shown to be effective for NDI by earlier authors (Stampfer *et al.* 1984). For our third study, we also took dates of death as reported by USRDS to overcome this limitation. USRDS uses the Social Security Administration database to ascertain vital status. There is growing literature on the accuracy of data in the 2728 CMS forms. The accuracy varies greatly depending on the field being studied (Layton *et al*, 2010; Merkin *et al*, 2007). However, based on personal communication with USRDS representatives (Beth Forrest)

we are confident that the fields we used are reasonably accurate. In addition, for the current analyses, we used the most updated field or the one with the least missing for each variable used from the various data sets available from the USRDS.

An important limitation is the overall healthy worker effect in this rather young cohort, in which only about 6% have died. Furthermore, internal comparison between those with low blood lead and those with higher blood lead are made difficult by the much higher length of follow-up for the higher blood lead categories, for which the healthy worker effect would be expected to be correspondingly less. This is especially true for non-malignant causes, which are more susceptible to the healthy worker effect.

Yet another limitation is that we did not have data on race for 69% of our cohort, and we these we classified as white race in study 1. Among those with known race, 80% were white. We can presume that most non-whites in our study population were black, as is the case for non-whites in the US as a whole. For lung cancer, the black male rate over the last 20 years has been 87/100,000, compared to a white rate of 68/100,000, and the combined male rate has been 69/100,000 (CDC, cdc.wonder.gov). In our study population, we assumed overall a population of 6% non-white, where it is likely that the true non-white population was 20%. Combining these data, it is likely that we have underestimated our US population lung cancer rate by about 4%, and hence we have over-estimated our lung cancer SMRs by about 4%. This is only a small amount, and our basic conclusions regarding lung cancer are unchanged.

Non-whites are known to have much higher rates of ESRD incidence than whites. For example, age and gender-adjusted USRDS ESRD incidence rates (per 100,000) for African-Americans and whites in 2010 in the US were 92.4 and 27.5 respectively (http://www.usrds.org/reference.aspx, accessed June 9, 2013). Furthermore, in those with known race based on state data (31% of the cohort), race (white/non-white) was a confounder, in that it was strongly related to ESRD incidence, and also weakly related to BL category (higher BL categories had higher percent non-whites, 15% in the lowest BL category, 18% in the highest). Thus, the fact that 69% of our cohort was missing data on race created a potential for bias. Furthermore, because race was a required field to run the NIOSH life table, we were unable to use LTAS without assigning some value for race (white/non-white) to those missing race. It should be noted, however, that we had race data on all ESRD cases from the USRDS. As a result we had to use imputation techniques for our second study. Inspite of this approach, these methods are far from being perfect and the AUC for the imputed values was 0.69, or a moderately good prediction.

Future Directions

In the future, we intend to run a nested case-control study to gather work history information and/or information on possible confounding variables such as smoking. Since this is a relatively young cohort we also intend to follow-up with further matching with the NDI and USRDS to ascertain patterns of association among lead exposure in categories with other outcomes.

APPENDIX 1: LEAD EFFECTS ON THE BODY: PATHOPHYSIOLOGY

Once lead enters the body, there are multiple pathways through which lead might produce toxic effects and eventually disease.

1. OXIDATIVE STRESS: Chronic lead exposure has been associated with disruption of the pro- and anti-oxidant balance in the body. Studies in animals and later human studies have shown the effect of reactive oxygen species (ROS) on health of those exposed to lead. However, these studies are not conclusive. Multiple mechanisms have been put forth (Kopp *et al.* 1988, Apostoli *et al.* 2004, Zysko *et al.* 2004, Zawadzki *et al.* 2006) based on these studies including

a. increased synthesis of reactive oxygen species and functional NO deficiency,

b. changes in physiology of the muscular and endothelial layers induced by direct interaction of lead ions with walls of blood vessels,

c. stimulation of the sympathetic systems

d. depressed vascular and increased renal beta receptor densities,

e. elevated catecholamine and endothelin production,

f. reduction in vasodilatatory prostaglandins and elevation of vasoconstrictive prostaglandins,

g. disturbances in or activation of the renin–angiotensin–aldosterone system

h. Abnormalities in kallikrein–kinin.

i. Elevated levels of homocysteine,

j. Elevation of inflammatory agents: C-reactive protein (CRP), pro-inflammatory interleukins and tumour necrosis factor-α (TNF-α).

Reactive oxygen species (ROS) have been shown to have a role in development of hypertension, (Touyz 2003, Madamanchi *et al.* 2005, Singh *et al.* 2006, Vokurkova *et al.* 2007, Victor *et al.* 2009, Touyz *et al.* 2011), kidney disease (Pohlman *et al.* 2000, Briet *et al.* 2012, Brodsky *et al.* 2012, Vostalova *et al.* 2012), cancer (Valko *et al.* 2005, Valko *et al.* 2006, Reuter *et al.* 2010), cerebrovascular (Allen *et al.* 2009, Chen *et al.* 2011, De Silva *et al.* 2011, Pradeep *et al.* 2012) and cardiovascular disease (Ferrari *et al.* 1991, Ercal *et al.* 2001, Bandyopadhyay *et al.* 2004, Pashkow 2011, Vural *et al.* 2012), mainly as a consequence of imbalance between production of ROS from mitochondria in cells and anti-oxidant activity in the body. Multiple studies, both animal and human, have demonstrated the effect of lead exposure on increasing production of ROS (Vaziri *et al.* 2003, Zhan *et al.* 2004, Farmand *et al.* 2005, Jomova *et al.* 2011), as well as impaired elimination of ROS by anti-oxidants (Lawton *et al.* 1991, Sugawara *et al.* 1991, Bechara 1996, Chiba *et al.* 1996, Vaziri *et al.* 1999a, Flora *et al.* 2000, Hsu *et al.* 2002, Han *et al.* 2005). Further credence to the lead affecting blood levels of ROS has been lent by studies demonstrating decrease in blood lead levels of ROS among lead-exposed when administered anti-oxidants (Mohammad *et al.* 2010, Jackie *et al.* 2011). Other studies that simultaneously administered lead and high doses of anti-oxidants found no increase in ROS (Chaurasia *et al.* 1997, Batra *et al.* 1998). Anti-oxidants like vitamin E (Ferrari *et al.* 1991, Chaurasia *et al.* 1997, Vaziri *et al.* 1999a, Vaziri *et al.* 1999b, Patra *et al.* 2001, Hsu *et al.* 2002, Flora *et al.* 2003, Kaczmarek-Wdowiak *et al.* 2004, Marchlewicz *et al.*

2004, Valko *et al.* 2005, Valko *et al.* 2006, Flora *et al.* 2007, Jomova *et al.* 2011), vitamin C (Ferrari *et al.* 1991, Hsu *et al.* 1998, Dawson *et al.* 1999, Marques *et al.* 2001, Patra *et al.* 2001, Hsu *et al.* 2002, Flora *et al.* 2003, Kaczmarek-Wdowiak *et al.* 2004, Marchlewicz *et al.* 2004, Zhan *et al.* 2004, Shalan *et al.* 2005, Valko *et al.* 2005, Valko *et al.* 2006, Flora *et al.* 2007, Mohammad *et al.* 2010, Jomova *et al.* 2011), vitamin B6 (McGowan 1989), zinc (Kromhout *et al.* 1985, Hashmi *et al.* 1989a, Hashmi *et al.* 1989b, Sroczynski *et al.* 1990, Murata *et al.* 1991, Staessen *et al.* 1992, Skoczynska *et al.* 1993, Skoczynska *et al.* 1994, Cocco *et al.* 1995, Staessen 1995, Staessen *et al.* 1996, Boscolo *et al.* 1997, Batra *et al.* 1998, Boscolo *et al.* 2000, Hsu *et al.* 2002, Siddiqui *et al.* 2002, Flora *et al.* 2003, Kasperczyk *et al.* 2004, Zhan *et al.* 2004, Carta *et al.* 2005, Kasperczyk *et al.* 2005, Muzi *et al.* 2005, Valko *et al.* 2005, Patil *et al.* 2006a, Patil *et al.* 2006b, Poreba *et al.* 2010a, Jomova *et al.* 2011, Poreba *et al.* 2011a), selenium (Rastogi *et al.* 1976, Flora *et al.* 1983, Othman *et al.* 1998, Hsu *et al.* 2002, Zhan *et al.* 2004, Valko *et al.* 2006, Alatise *et al.* 2010), N-acetylcysteine (Ferrari *et al.* 1991, Ercal *et al.* 1996, Flora *et al.* 2004), methionine (Patra *et al.* 2001), and taurine (Wright *et al.* 1986, McGowan 1989, Gurer *et al.* 2001, Flora *et al.* 2007) have been found to decrease toxic effects of lead exposure. Chelation of blood lead also led to reversal of lead induced free radical generation (Flora *et al.* 2007, Flora *et al.* 2008). ROS, such as hydroxyl radicals, superoxide anions and lipid radicals, have been implicated in the chemical modification of macromolecules, thereby causing changes in the cell membranes of cells and hence impairment of organ function (Valko *et al.* 2005, Valko *et al.* 2006, Jomova *et al.* 2011). The primary sources of anti-oxidative activity in the body include enzymes, like

manganese superoxide dismutase [Mn-SOD] (Ito *et al.* 1985, Ferrari *et al.* 1991, Chiba *et al.* 1996, Vaziri *et al.* 2003, Zhan *et al.* 2004, Farmand *et al.* 2005, Han *et al.* 2005, Jackie *et al.* 2011, Jomova *et al.* 2011), copper/zinc superoxide dismutase [Cu/Zn SOD] (Farmand *et al.* 2005), glutathione reductase, glutathione peroxidase , and catalase [CAT]) (Hashmi *et al.* 1989b, Ferrari *et al.* 1991, Sugawara *et al.* 1991, Sandhir *et al.* 1994, Bechara 1996, Chiba *et al.* 1996, Cadenas *et al.* 2000, Flora *et al.* 2003, Vaziri *et al.* 2003, Kasperczyk *et al.* 2004, Zhan *et al.* 2004, Farmand *et al.* 2005, Han *et al.* 2005, Valko *et al.* 2006, Korge *et al.* 2008, Vassalle *et al.* 2008, Kasperczyk *et al.* 2009, Zhang *et al.* 2009, Jackie *et al.* 2011). Other factors leading to increased ROS levels are impaired regulation of calcium channels (Bhunia *et al.* 1997), altered activity of tyrosine kinases and proteins, changes in NO synthase activity (Valdes Bolanos), especially endothelial NO synthase (Qian *et al.* 1999, Munzel *et al.* 2005, Grobe *et al.* 2006, Valko *et al.* 2007, Schulz *et al.* 2008, Forstermann 2010), increased concentrations of inflammatory markers and functional changes in immunological molecules (CRP, cytokines, TNF, homocysteine) (Qian *et al.* 1999, Cai *et al.* 2000, Elahi *et al.* 2009), and altered transcription factor activity, especially peroxisome proliferators activated receptor [PPAR] (Keller *et al.* 1993, Mattson *et al.* 2004, Reuter *et al.* 2010) and nuclear factor kappaB [NFκB](Keller *et al.* 1993, Gius *et al.* 1999, Jeay *et al.* 2003, Mattson *et al.* 2004, Reuter *et al.* 2010).

2. ABNORMALITIES IN BLOOD LIPID LEVELS: Data from multiple animals studies indicated that lead ingestion can lead to increased blood lead levels. However, human data are scarce and contradictory. The proposed mechanisms by which lead

exposure leads lipid abnormalities (Lawton *et al.* 1991, Boadi *et al.* 1992, Skoczynska *et al.* 1994, Likholat *et al.* 2000a, Likholat *et al.* 2000b, Pillai *et al.* 2002, Stojek *et al.* 2003, Kaczmarek-Wdowiak *et al.* 2004, Dursun *et al.* 2005, Kasperczyk *et al.* 2005, Adegbesan *et al.* 2007, Zhang *et al.* 2009) are:

a. Changes in polyunsaturated fatty acid (PUFA) metabolism,

b. Activation of lipid synthesis,

c. Induction of lipid peroxidation,

d. Mutations of arterial wall cells and

e. Inhibition of anti-oxidative enzymes (vide supra)

Abnormal blood lipid levels (elevated LDL and total cholesterol, and low HDL levels) are known risk factors for cardiovascular disease (CVD) (Boden 2000). Studies among rats fed on 0.1% lead acetate mixed in drinking water showed a significantly increased concentration of total cholesterol(Hashmi *et al.* 1989a, Hashmi *et al.* 1989b). Skoczynska *et al*. (2007) and Doroszko *et al*. (2008) independently demonstrated a positive association between blood lead levels and serum LDL concentrations in humans (Skoczynska *et al.* 2007, Doroszko *et al.* 2008). Occupational exposure to lead was associated with elevated levels of LDL cholesterol, triglycerides and total cholesterol, and decreased levels of HDL cholesterol (Gatagonova 1994). A study of lead workers found significantly increased levels of arachidonic acid (AA), a cholesterol pathway product, as compared to matching healthy controls in the RBC of the lead-exposed subjects (Osterode *et al.* 2000). Novel CVD risk factors, such as elevated blood levels of triglycerides, the presence of small, dense LDL, and elevated concentration of lipoprotein

(a) (Lp-a), increased concentrations of endothelial dysfunction markers, and elevated levels of pro-inflammatory factors have been associated with lead exposure in human studies (Skoczynska *et al.* 1993, Schwartz 1995, Onat *et al.* 2001, Onat *et al.* 2003, Onat 2004, Onat *et al.* 2005, Onat *et al.* 2007).

3. CHANGES IN ARTERIAL BLOOD PRESSURE: Increase in arterial pressure has been widely accepted as a major risk factor in the development of heart disease (Rosendorff 2007). Increases in blood pressure have been demonstrated in experimental models, and these results have been replicated in human studies. Lead exposure has been associated with the following:

a. increases in pulse pressure

b. Higher levels of zinc protoporphyrin (Poreba *et al.* 2010b).

c. Increased arterial stiffness

d. Increased carotid intima-media thickness (Poreba *et al.* 2010a, Poreba *et al.* 2010c, Poreba *et al.* 2011b).

4. CHANGES IN IMMUNE FUNCTION: Lead exposure has been associated with altered immune function in animal and human studies. However, these data are scarce.

a. Affects lymphocytes and macrophages interfering with the humoral response and cellular immune response natural killer cells (NK) and endothelial cells (Krocova *et al.* 2000).

b. Modify the activity of Langerhans cells and dendritic cells in the skin (Aiba *et al.* 1997).

c. Causes target cells to release pro-inflammatory mediators and, chemotactic and pro-coagulative factors mediated through increased production of ROS and interfering with anti-oxidative enzyme activity (vide supra).

5. CHANGES IN AUTONOMIC NERVOUS SYSTEM (ANS): Lead exposure has been associated with decreased HRV, due to decreased parasympathetic tone rather than increased sympathetic tone (Murata *et al.* 1991, Murata *et al.* 1995, Andrzejak *et al.* 2004, Gajek *et al.* 2004, Poreba *et al.* 2011c). However, studies may and may not show these effects, and these results are not conclusive.

6. CHANGES IN ECG: Studies on the effect of lead exposure on heart rhythm and electrocardiographic patterns have not shown consistent patterns. The following changes have been seen.

a. Tachycardia

b. Bradycardia and shortening of P-Q interval (Kosmider *et al.* 1961),

c. Rhythm disorders,

d. Prolongation of P wave, QRS complex, and QT interval,

e. Denivelations of ST segment

f. Atrio-ventricular and intra-ventricular conduction disorders.

g. Decreased heart rate variability (HRV)

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