METAL EXPOSURES AND KIDNEY OUTCOMES IN LEAD WORKERS

by

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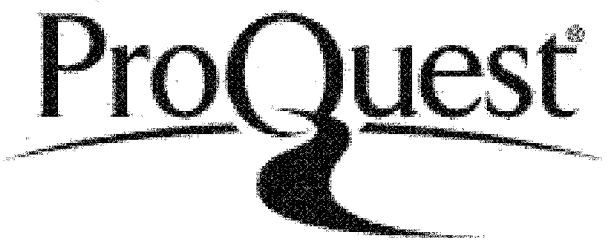

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ABSTRACT

Chronic kidney disease (CKD), which includes end stage renal disease (ESRD) and the stages of renal dysfunction that precede it, is a growing public health problem. For example, in the US, an estimated 13.1% of the adult population has CKD[1] and the number of cases of ESRD is expected to rise from approximately 485,000 in 2005 to 785,000 by 2020. CKD is also an economic concern, where 2005 annual Medicare expenditures for ESRD alone approached $20 billion.[2] Comprehensive primary prevention strategies to reduce the burden of CKD are essential. Strategies have traditionally focused on well known risk factors for CKD such as diabetes mellitus (DM) and hypertension (HTN).[3] In contrast, the impact of occupational and environmental nephrotoxicants, such as heavy metals, on CKD risk has received little attention.

Epidemiologic studies have established associations between nephrotoxic effects and a number of metals, including lead and cadmium.[4-6] Other metals, such as antimony, thallium, and uranium, are considered potential nephrotoxicants, as deleterious effects on the kidney have been observed in animal studies and acute human poisonings, yet very few human studies exist to evaluate evidence of nephrotoxicity at occupational and environmental exposure levels. Even fewer studies have examined the impact of co-exposure to these metals in workers with occupational exposure to known nephrotoxicants (e.g. lead). Hence, the objectives of this cross-sectional study were to evaluate associations between urine concentrations of antimony, thallium, and uranium and kidney outcomes using data from a cohort of Korean lead workers.

First, we performed a cross sectional analysis of 684 current and former lead workers to evaluate associations of occupational exposures to antimony and thallium and kidney outcomes as well as evaluate the impact of environmental cadmium exposure on those associations. Median levels of urine antimony were over eight times greater in current workers whereas median thallium concentrations were relatively similar between current and former workers. After

adjustment for lead dose, urine creatinine, and kidney risk factors, we found that higher *ln*-urine thallium, was associated with higher serum creatinine- and cystatin-C-based estimates of glomerular filtration rate (eGFR), a pattern opposite that which is traditionally expected in nephrotoxicity. These associations remained significant after adjustment for antimony and cadmium. Antimony associations with kidney outcomes were attenuated by thallium and cadmium adjustment; associations of both antimony and thallium with N-acetyl-β-D-glucosaminidase (NAG), a biomarker indicative of kidney proximal damage, were attenuated by cadmium.

Second, we performed a cross-sectional analysis of the associations between environmental uranium exposure and kidney outcomes in the same population of 684 current and former lead workers. After multivariate adjustment, including lead dose and urine thallium and cadmium, higher *ln*-urine uranium was associated with lower measured creatinine clearance and higher NAG, a pattern consistent with that traditionally expected in nephrotoxicity. However, these associations were observed only in models using urine creatinine-based methods to correct for urine dilution. In contrast to thallium and cadmium, uranium was not associated with any other eGFR measures.

These results suggest that interpretation of low-level urine metal values may be more complex than previously appreciated. Several potential hypotheses were considered to explain the novel results of our study. The paradoxical direction of thallium associations with both serum and cystatin-C-based glomerular filtration measures is consistent with a kidney function effect such as reverse causality or thallium-related hyperfiltration. However, neither hypothesis is as relevant for the antimony and cadmium associations observed with serum creatinine- but not cystatin-C-based filtration measures. A statistical association related to the use of creatinine in both exposure and outcome metrics is a consideration for these findings. The striking change in urine creatinine associations when a significant metal is in the model lends support for this hypothesis. The fact that urine uranium levels were significantly associated with measured

creatinine clearance and NAG only when urine creatinine was included in the regression model also suggests that these results may be, in part, due to a biostatistical association. However, given the different patterns in associations between metals, unique metal-specific mechanisms, such as metal-protein binding affecting excretion [7] must also be considered. Additional research is needed to fully understand the mechanism(s) underlying our results. In such work, kidney research that uses both creatinine- and non-creatinine-based outcomes should consider exploration of non-creatinine based methods of adjustment for urine dilution as well as non-urine based exposure measures (e.g. blood levels).

Novel results were also observed in the multiple metals analysis which revealed attenuation of observed associations by other metals, in particular cadmium. These results suggest that single metal exposure analyses may increase the potential for inaccurate risk conclusions.

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PREFACE

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ABBREVIATIONS

BLL	blood lead level
BMI	body mass index
BP	blood pressure
Cd	cadmium
CI	confidence interval
Creat.	creatinine
CV	coefficient of variation
CKD	chronic kidney disease
CYSeGFRc	combined serum creatinine/cystatin C-based eGFR
CYSeGFRm	multi-variable Cystatin C-based eGFR
CYSeGFRs	single Variable Cystatin C-based eGFR
DBP	diastolic blood pressure
DM	diabetes mellitus
DRC-ICP-MS	dynamic reaction cell inductively coupled plasma mass spectrometer
eGFR	estimated glomerular filtration rate
ESRD	end stage renal disease
GFR	glomerular filtration rate
HNO3	nitric acid
HTN	hypertension
ICP-MS	inductively coupled plasma–mass spectrometer
ln-	natural logarithm
IQC	internal quality control
m2	meters squared

MDL	method detection limit
MDRD	Modification of Diet in Renal Disease
mg/dL	milligrams per deciliter
mg/L	milligrams per liter
mL	milliliter
mL/min	milliliters per minute
MLR	multiple linear regression
NAG	N-acetyl-β-D-glucosaminidase
NKF	National Kidney Foundation
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
QC	quality control
Sb	antimony
SBP	systolic blood bressure
SD	standard deviation
Tl	thallium
U	uranium
XRF	X-ray flourescence
µg/g	micrograms per gram
µg/L	micrograms per Liter
µmoL	micromoles per liter

Chapter 1: INTRODUCTION

Chronic kidney disease (CKD), which includes end stage renal disease (ESRD) and the stages of renal dysfunction that precede it, is a substantial public health problem. For example, an estimated 13.1% of the U.S. adult population has CKD.[1] Furthermore, in 2005, there were approximately 485,000 cases of ESRD in the U.S., a number that is expected to rise 60% by 2020. CKD is also an economic burden, with the 2005 annual Medicare expenditures for ESRD alone reaching $20 billion.[2]

Comprehensive primary prevention strategies are essential in lowering the health and economic burden of CKD. Two well known risk factors for CKD are diabetes mellitus (DM) and hypertension (HTN).[3] Hence, current CKD prevention strategies have focused on better disease management, such as diet and lifestyle modification. In contrast, the impact of occupational and environmental exposures on CKD risk has received little attention. Metals are an important group of chemicals for consideration in this regard. A number of metals are known or suspected renal toxicants, ubiquitous in the environment and present in biologic specimens of most US residents. While exposure to metals in the U.S. and other developed countries is conventionally perceived as decreasing, it remains a global issue, especially in less developed countries where metals are widely produced and used in manufacturing processes (e.g. electronics) and in products, such as leaded paint,[8, 9] that are now banned in developed countries. Improper disposal of metal-containing products will continue to be an important source of exposure. Additionally, there are rising concerns that the exponential development and global application of metal based nanomaterials will introduce new health risks.[10]

Inorganic lead, which has been shown to increase the risk for initiation and/or progression of CKD even at current environmental levels,[11] is the most recognized nephrotoxicant among the metals. Cadmium is also a nephrotoxic metal at occupational and high

environmental levels of exposure.[12, 13] Data on nephrotoxic effects of most other metals are more limited. However, there is emerging evidence that other metals present in occupational and environmental settings may adversely affect renal function. Additionally, metals with long half-lives in the body (e.g. lead and cadmium) represent a chronic endogenous exposure source. Thus, risk from nephrotoxicants that accumulate is greatest in aging populations where the prevalence of risk factors, such as HTN and DM, is also highest. Increased CKD risk from multiple established risk factors is well recognized. However, humans are also commonly exposed to mixtures of metals. The nephrotoxic and potential interactive effects of exposure to multiple metals are relatively unknown.

In order to assess the nephrotoxic potential for a range of metals, we analyzed data on a selected panel of urine metals from a longitudinal cohort study focused primarily on the risk for nephrotoxicity associated with lead and environmental cadmium exposure in 684 Korean lead workers. Factors considered in determining which metals to analyze for the proposed study were: evidence of nephrotoxicity, structural/mechanistic similarities to existing nephrotoxicants, and exposure potential. This study tests the hypothesis that urine levels of antimony, thallium, and uranium are associated with kidney function measures. We also evaluated the impact of cadmium on these associations. Traditional clinical measures of kidney function were assessed by serum creatinine, serum cystatin c, calculated creatinine clearance[14], measured creatinine clearance, and serum creatinine- and cystatin-C-based estimated glomerular filtration rates (eGFR).[15] An early biologic effect marker, N-acetyl-β-glucosaminidase (NAG), was investigated as an early indicator of proximal tubular damage.

The specific aims of this cross-sectional study were to:

1. examine associations between clinical kidney outcome measures and urine concentrations of antimony, thallium, and uranium;
2. examine associations between a potential biomarker of early nephrotoxic effects (N-acetyl-β-glucosaminidase), and urine concentrations of the above metals; and

3. evaluate the impact of urine cadmium on these associations.

To achieve these specific aims, we focused our analyses separately according to primary source of exposure – occupational or environmental.

We first performed a cross sectional analysis of 684 current and former lead workers to evaluate associations between occupational exposures to antimony and thallium and kidney outcomes as well as to evaluate the impact of environmental cadmium exposure on those associations. Findings from these analyses are reported in Chapter 3: Associations of Multiple Metals with Kidney Outcomes in Lead Workers (Manuscript I)

Second, we performed a cross-sectional analysis of the same 684 current and former lead workers to examine associations between environmental uranium exposure and kidney outcomes as well as to evaluate the impact of environmental cadmium exposure on those associations. Findings for these analyses are reported in Chapter 4: Associations of Environmental Uranium Exposure with Kidney Outcomes in Lead Workers (Manuscript II)

Chapter 2: BACKGROUND AND SIGNIFICANCE

Public Health Burden of Kidney Disease

Chronic kidney disease (CKD), which includes end stage renal disease (ESRD) and the stages of renal dysfunction that precede it, is a considerable public health concern. For instance, the prevalence of CKD is estimated to be 13.1% in the U.S. adult population according to a recent analysis of National Health and Nutrition Examination Surveys (NHANES) data.[16] Furthermore, in 2005, approximately 485,000 Americans were classified as having ESRD. This number is expected to increase approximately 60% by 2020.[2] CKD is also an economic burden, with the 2005 annual Medicare expenditures for ESRD alone reaching $20 billion.[2] The need to identify novel, modifiable risk factors for CKD is increasingly urgent. Environmental nephrotoxicants are considered to be one such novel risk factor for CKD.

Metals as Nephrotoxicants

Multiple chemicals have been identified as nephrotoxicants based on occupationally or environmentally exposed humans and/or animal models.[17-19] Metals are an important chemical group in this regard as occupational and environmental studies have shown associations between nephrotoxic effects and a number of metals, including lead and cadmium.[4-6] Other metals may also be nephrotoxic, but, for most, there has been relatively little occupational or environmental research. We were fortunate to have access to demographic and biologic data and stored urine samples from a cohort of Korean lead workers in a study that originally focused on the nephrotoxicity of lead and cadmium. We were able to measure multiple trace metals in urine via an inductively coupled plasma mass spectrometry (ICP-MS) assay[20] from the stored urine samples in order to examine associations between kidney outcomes and additional metals, namely antimony, thallium, and uranium, in the Korean lead worker cohort.

The following criteria were considered in prioritizing candidate urinary metals that merited additional study:

1) Weight of evidence. The metal should be a suspected nephrotoxicant based on chronic exposure data in animals and/or occupationally or environmentally exposed humans. However, because of the lack of chronic low level exposure data, we considered the findings of studies of acute high dose exposures. In the absence of adequate human or animal data, metals with structural or mechanistic similarities to established nephrotoxicants were also considered.

2) Exposure potential. Metals that pose ubiquitous exposures put a greater proportion of the population at risk. For instance, urine antimony, thallium, and uranium levels were detectable in 86.7%, 99.9%, and 92.3% respectively, of NHANES 05-06 participants.[21]

3) Half-life. Metals with long half-lives that accumulate in the body are of greater concern because they can result in ongoing sources of endogenous exposure. This increases risk particularly in older age when other CKD risk factors, such as hypertension (HTN) and diabetes mellitus (DM),[22, 23]are more prevalent.

4) Biomarkers of exposure. The New York State Department of Health Trace Elements Laboratory has the capacity to measure multiple trace metals in urine via an inductively coupled plasma mass spectrometry (ICP-MS) assay.[20] This method is able to obtain rapid and accurate measurements with sensitivity sufficient to quantify environmental level exposures to multiple metals in urine.[24]

The considerations supporting the selection of antimony, thallium, and uranium for additional study are described below in the context of these criteria:

Antimony

Antimony is released into the environment during mining and manufacturing processes as well as from coal fired power plants and incinerators. One of the most important uses of antimony is as an alloy in lead storage batteries, leading to the potential for increased exposure in occupational populations such as ours. For example, Table 2-1 demonstrates that the median urinary antimony level in our population is nearly twice as high as the corresponding 2005-2006 NHANES level.[21] Industrial releases are eventually deposited into soil or sediment where unbound antimony is taken up by plants or animals; therefore, ingestion of contaminated food is generally the main source of environmental exposure.[21]

Little research has been conducted on the absorption of antimony in humans, but the absorption rate is thought to be a function of solubility and/or size (e.g. respiratory absorption is more likely with smaller particles). Additionally, at least some forms are absorbed in the gastrointestinal (GI) tract. For example, the absorption of antimony tartrate and antimony trichloride has been estimated to be 2% to 7% in animals.[25] Once absorbed, following either parenteral or acute and chronic oral exposure, antimony is distributed to the thyroid and adrenal glands, but also, to a lesser extent, the kidney and liver. Urine is the major route of excretion for pentavalent antimony, while feces is the major route for trivalent antimony. Urine antimony may be considered a biomarker of recent antimony exposure as the half-life estimates range from several hours for parenteral therapeutic agents to 4 days in a study of lead battery workers.[26]

Relatively little research on renal toxicity has been conducted on any antimony compounds, especially at environmental exposure levels. Tubular damage in humans has been reported to be a potential adverse effect of the parenterally administered anti-parasitic agent sodium antimony gluconate.[27] Tubular dilation and degeneration of the tubular epithelium has also been observed in several animal species after acute, but not intermediate or chronic inhalation exposure to antimony trisulfide or stibine gas, which are antimony compounds commonly used in industry.

In the absence of renal outcomes data, adverse effects in organs that may be subject to similar mechanisms of toxicity, such as peripheral arteries, support the need for investigation of antimony as a potential nephrotoxicant. In this regard, a recent analysis of NHANES data that identified antimony as a potential risk factor for peripheral arterial disease[28] (PAD) is relevant. Incidentally, the risk of morbidity for patients with CKD and PAD has been found to be higher than for patients with either disease alone.[29]

Thallium

Thallium is often used as an alloy and is a byproduct in the smelting of metals such as lead. Therefore, thallium is another metal for which there is potential for occupational exposures in the study population. This is demonstrated in Table 2-1 which shows that the median urine thallium values for the study population were greater than those for adults in the 2005-06 NHANES population. Thallium has not been mined or refined in the US since 1984, and it is also restricted from pesticide and cosmetic uses however, such restrictions are not present globally.[30] Electronics manufacturing accounts for the largest consumer use in the US. Industrial use of thallium may increase in the future because it functions as a catalyst in the emerging field of organic chemical synthesis.[27] Exposure to fine particulate matter released by these facilities is likely to be the most significant source of exposure in the general population. Ingestion of thallium through contaminated drinking water or plants that have absorbed thallium from nearby coal burning or metal smelting facilities is also possible. Additionally, thallium is a bioaccumulative metal and elevated concentrations in trout from Lake Michigan have generated serious concern about adverse health risks from contaminated fish consumption, especially in the Great Lakes. Consequently, it has been suggested that the exclusion of thallium from fish consumption advisories may be a serious oversight and further investigation into potential for chronic thallium poisoning has been recommended.[31, 32]

Thallium is rapidly absorbed via all exposure routes, and is almost completely absorbed in the GI and respiratory tracts. In cases of fatal human poisonings, thallium has been found to be

widely distributed throughout the body, with the highest concentration in the kidneys. Acute and chronic animal exposure studies indicate a similar pattern of distribution.[27] The half life of thallium in humans is estimated to be several days regardless of organ.[27, 33] The elimination of absorbed thallium varies by time. The majority is initially eliminated in feces, and a lesser proportion in urine within ensuing weeks. Most of the remaining body burden is slowly eliminated in hair.[27]

Thallium is best known as a potent human neurotoxin and is considered to be more acutely toxic than other relatively well studied neurotoxic metals such as lead, cadmium, and mercury.[31] Data on renal effects in animals or humans are lacking, especially at chronic environmental exposure levels. Aminoaciduria;[34] decreased urea clearance and specific gravity, albuminuria and;[35] tubular necrosis and acute renal failure[36] have been reported in cases of acute high dose ingestion in humans. Tubular necrosis with glomerular hyalinization[37, 38] and decreased GFR[39, 40] have also been observed in animal studies of acute exposures. Decreased GFR may be a result of the observed destruction of the ascending limb of the loop of Henle.[39]

Uranium

Uranium is a heavy metal naturally found in rocks and soil.[41] Various uranium compounds can also be released into the environment from mining and industrial processes related to its use as a power source and in a variety of products such as glass tint, glazes, and military munitions. Exposure in the general population occurs mainly through ingestion of uranium adsorbed onto food grown in contaminated soil, particularly root vegetables, or in drinking water. [42] Exposure to natural uranium via contaminated ground water is a global concern as elevated levels have been detected in a wide range of geographic areas such as eastern and southwest United States, Canada, Scandinavian countries, central Australia, India, and South Korea.[41, 43, 44] The main industrial use of uranium is as a power source for nuclear reactors,[43] a use which is expected to rise with increasing energy demand.[45] Therefore, exposure potential related to

uranium mining activities and nuclear power plant emissions will continue to be an important consideration.

Ingested uranium is generally poorly absorbed (< 3.2 % in humans) with the greatest absorption occurring in a fasting state or in forms with greater water solubility.[27, 41, 46] An estimated 66% of the uranium dose that enters the human bloodstream is excreted into urine within 24 hours and nearly 90% within a month.[47] The remainder of absorbed uranium is primarily distributed to bone, and to a lesser extent, the kidneys and liver. The half life in bone may range from months to years. Therefore, urine uranium is considered to be a recent exogenous dose measure with a small contribution from cumulative endogenous sources.[21, 27, 42, 46]

Nephrotoxic effects, including alterations in glomerular and proximal tubule structure and function, have been observed in acute and sub-acute animal exposure studies using several different uranium compounds. Evidence of proximal tubule damage has been reported in the relatively limited number of chronic exposure studies in animals.[48] Glomerular and proximal tubule damage have also been reported in several cases of acute high dose exposures in humans, such as in industrial accidents.[49] Proximal tubule damage has been the primary effect found in a number of epidemiological studies.[41] These nephrotoxic effects associated with uranium exposure have been attributed to the chemical rather than radiological toxicity of uranium.[41, 42] The US EPA has established a Maximum Contaminant Limit (MCL) of 30 μg/L of uranium in drinking water based on renal effects observed in animal ingestion studies.[50]

Multiple Metal Exposures

Nephrotoxicity associated with mixed metal exposures, and its contribution to the overall incidence of CKD, has been inadequately studied, despite the fact that humans are commonly exposed to mixtures of metals. There are concerns that co-exposures to metals with similar toxicokinetic (e.g. half lives) and toxicodynamic (e.g. proximal tubular damage) properties will

produce additive or multiplicative effects on the kidney. Emerging evidence indicates that these relationships deserve research and public health consideration. For example, a recent analysis of 1999-2006 NHANES data found that the odds of having both albuminuria and reduced eGFR were four-fold higher in participants in the highest quartiles of both blood cadmium and lead compared to those in the lowest.[51] Thus the current study expanded our knowledge of nephrotoxic effects of mixed metals by considering co-exposures to lead, cadmium, and three additional metals that share some structural and mechanistic properties with lead and cadmium.

Co-exposure

The study population was drawn from a cohort with known occupational lead exposure in which cumulative and current lead dose have been associated with change in kidney function.[12] A majority of the participants were employed in lead acid battery manufacturing plants where they were also occupationally exposed to antimony because it is used in the battery manufacturing process. However, environmental exposures are also relevant to this study population as evidenced by the association of increased levels of NAG, a biomarker of proximal tubule effect, with environmental cadmium exposure as shown in a previous cross sectional analysis of the cohort study.[12] As mentioned above, urine metal levels in this study population are higher than those found in the 2005-2006 NHANES populations in the case of all three study metals (Table 2-1).

Since lead accumulates in bone and has a half-life ranging from 10-30 years, chronic endogenous exposure to lead [52, 53] may increase the risk for CKD with age [12] as with HTN and DM.[54] Uranium, which is also ubiquitous but often unrecognized, may also accumulate in bone where the half-life can range from months to years depending on the amount absorbed.[42] Therefore, this shared property of lead and uranium that can result in ongoing endogenous co-exposures represents an important rationale in the selection of uranium for additional study.

Table 2-1. Comparison of Korean Lead Worker Cohort and NHANES 2005-2006 urine metal levels

	[redacted]	[redacted]	[redacted]	[redacted] Population ([redacted] percentile)
Antimony, (μg/g creatinine)	0.06	0.25	0.29	10.21
Thallium, (μg/g creatinine)	0 .15	0.37	0.36	0.97
Uranium, (μg/g creatinine)	0.006	0.026	0.07	0.53

Similar Mechanisms of Action

The proximal tubule appears be the main site of metal-induced nephrotoxicity, including that caused by lead and uranium.[55, 56] This is thought to occur as a result of the tubular re-absorption of metal-protein complexes that eventually lead to epithelial membrane damage.[19] Although the mechanisms of metal entry into the proximal tubule cells are not widely understood, recent studies indicate that entry by lead and cadmium occurs by the same processes because of structural similarities.[55] For example, lead and cadmium are both divalent cations that inhibit enzymes that contain sulfhydryl groups.[57] Oxidative stress, apoptosis, and necrosis appear to be common mechanisms of metal-induced intracellular toxicity for a number of metals, including lead, cadmium, and uranium.[55]

Less is known about nephrotoxic actions of antimony and thallium, however there is some evidence that these agents cause tubular damage.[27, 37, 38] Furthermore, both antimony and thallium bind with proteins and sulfhydryl groups.[25, 31, 39] Therefore, it is plausible that mechanisms of metal-induced toxicity are similar to those of lead, cadmium, and uranium because of their structural and mechanistic similarities.

Evaluation of Kidney Function

Clinical measures reflective of glomerular function are traditionally used to diagnose CKD. The range of traditional clinical measures we examined as kidney outcomes include: serum creatinine, serum cystatin c, calculated creatinine clearance[14], measured creatinine clearance, and serum creatinine and cystatin C-based estimated glomerular filtration rates (eGFR).[15] Of these, decline in the glomerular filtration rate (GFR) is considered the best measure of progressive renal damage.[58] Although the gold standard for GFR measurement is assessment of the urinary clearance of a filtration marker (e.g. ^{125}I-iothalamate)[59] from a 24 hour urine collection,[58, 60] cost and logistical factors prevent direct GFR measurement in most epidemiological studies. The use of equations based on traditional clinical measures, such as serum creatinine, to estimate GFR (eGFR) is the current preferred alternative. Increased serum creatinine levels indicate decreased kidney clearance of creatinine (i.e., decreased GFR) of which both reflect worse kidney function. In accordance with the current recommendations of the National Kidney Foundation (NKF), we used the widely accepted Modification of Diet in Renal Disease (MDRD) estimating equation[15] to predict GFR from serum creatinine.[61] Calculated creatinine clearance is another commonly used equation based on serum creatinine.[61] However, a limitation of using a serum creatinine-based eGFR is that levels are affected by factors other than the kidney. Creatinine is a product of muscle metabolism, thus muscle mass and dietary protein affect creatinine levels resulting in variation among population subgroups (e.g., lower levels in women compared to men) that are unrelated to kidney function. Therefore, we also used an estimating equation based on serum cystatin C, a cysteine protease inhibitor that is secreted by all nucleated cells,[62] thereby avoiding confounding with serum creatinine related to non-kidney factors.[63] Details of these estimating equations are shown in the methods section of the manuscripts below.

A major limitation in the use of traditional clinical measures to diagnose CKD progression is that abnormalities tend to appear relatively late in renal function decline. In recognition, the National Kidney Foundation (NKF) asserts that new biomarkers reflective of

renal damage prior to decreased GFR are needed.[64] N-acetyl-β-D-glucosaminidase (NAG), an early biological effect (EBE) marker of tubular damage, is of growing interest as the proximal tubule appears be the main site of metal-induced nephrotoxicity.[19] Urinary NAG, an enzyme normally present in proximal tubular cells, also indicates proximal tubular dysfunction and may indicate cellular necrosis.[60, 61] A previous cross sectional analysis of data from the lead worker cohort found positive associations with NAG for tibia lead and urinary cadmium. Cadmium is considered an environmental exposure in this population, illustrating the potential use of NAG as an EBE marker even at lower exposure levels.[12] Thus, assessment of NAG represents an opportunity to further investigate a more sensitive measure of renal function decline associated with metals before or in the earliest states of CKD.

Summary

CKD is a substantial current and future health and economic burden. The prevalence of well established risk factors, such as HTN and DM, are on the rise, thereby increasing the population at risk for CKD progression. The impact of occupational and environmental exposures on CKD risk has received little attention. Metals, such as lead and cadmium, are established nephrotoxicants. However, relatively little is known about the nephrotoxic effects of occupational or environmental exposures to other ubiquitous metals such as antimony, thallium, and uranium. Therefore, this study represents a novel opportunity to examine the potential associations between exposure to these metals, singly and in combination, and kidney outcome measures. Among these, the inclusion of NAG allowed for its evaluation as a possible early EBE marker of renal function decline associated with metal exposures.

Occupational and environmental exposures are modifiable risk factors, and identification of novel nephrotoxins could impact the prioritization of public health prevention strategies, allocation of resources, and development of policies related to exposure reduction efforts. Given

the increasing burden of CKD and the widespread exposure to metals which may alone or in combination be nephrotoxic, the potential implications of this study are considerable.

Chapter 3: ASSOCIATIONS OF MULTIPLE METALS WITH KIDNEY OUTCOMES IN LEAD WORKERS (MANUSCRIPT I)

Abstract

Background: Environmental exposure to multiple metals is common. A number of these have been reported to cause nephrotoxicity with acute and/or chronic exposure. However, few epidemiologic studies have examined the impact of metal co-exposure on kidney function.

Objectives: To evaluate associations of urine concentrations of antimony and thallium with kidney outcomes and to assess the impact of cadmium exposure on those associations in lead workers.

Methods: Multiple linear regression was used to examine associations between *ln*-urine thallium, antimony and cadmium levels with serum creatinine- and cystatin-C-based glomerular filtration measures, and *ln*-urine N-acetyl-β-D-glucosaminidase (NAG).

Results: In 684 participants, median urine thallium and antimony were 0.34 and 0.36 μg/g creatinine, respectively. After adjustment for lead dose, urine creatinine, and kidney risk factors, higher *ln*-urine thallium was associated with higher serum creatinine-based and higher cystatin-C-based estimates of glomerular filtration rate (eGFR); associations remained significant after adjustment for antimony and cadmium (beta-coefficient for serum creatinine-based eGFR = 5.2 mL/min/1.73 m^2; 95% confidence interval = 2.4, 8.0). Antimony associations with kidney outcomes were attenuated by thallium and cadmium adjustment; associations of both metals with NAG were attenuated by cadmium.

Conclusions: Urine thallium levels were significantly associated with both serum creatinine-based and cystatin-C-based glomerular filtration measures in a direction opposite that expected with nephrotoxicity. Given similarities to associations recently observed with cadmium, these results suggest that interpretation of urine metal values, at exposure levels currently present in the

environment, may be more complex than previously appreciated. These results also support consideration of multiple metal analysis approaches to decrease the potential for inaccurate risk conclusions.

Introduction

A number of metals are known or suspected occupational and/or environmental nephrotoxicants. Lead and cadmium are widely recognized in this regard [4, 65, 66]. Recent advances in analytical technology allowing measurement of multiple metals in urine samples have revealed that most US residents are exposed via the environment to a wide range of metals [67]. However, data on nephrotoxic effects of metals other than lead and cadmium are more limited and the impact of kidney exposure to multiple metals is relatively unknown. Populations with co-exposures may be at increased risk for deleterious kidney effects. Antimony and thallium are metals that are nephrotoxic in acute and subacute exposures [25, 68] and are common environmental exposures as evidenced by biomonitoring in the US general population [67]. Urine antimony and thallium were detectable in 86.7% and 99.9%, respectively, of NHANES 05-06 participants [69]. However, to our knowledge, no epidemiologic studies have examined associations of urine levels of either metal with kidney outcomes that include glomerular filtration measures. Therefore, to address the kidney impact of multiple metal exposures, we performed a cross-sectional analysis examining associations between urine antimony and thallium levels and kidney outcomes in 684 current and former lead workers in the Republic of Korea in whom cadmium associations with kidney function have also been analyzed [70].

Materials and Methods

Study Overview and Design

We performed a cross-sectional analysis of data from current and former inorganic lead workers who completed the fourth evaluation in a longitudinal study. Evaluations were performed

between April 8, 2004 and September 24, 2005. All participants provided written, informed consent. Participation in the study was voluntary, and workers were paid approximately $50 for their time and effort. The study protocol was approved by Institutional Review Boards at the SoonChunHyang University School of Medicine and the Johns Hopkins University Bloomberg School of Public Health.

Study Population

As previously described [12, 70, 71], participants in the initial cohort of this study were recruited between 1997 and 1999 (phase I) through medical surveillance programs at secondary lead smelters and plants that produced lead batteries, lead oxide, lead crystal, or radiators. The population is 100% Korean. In 2004, recruitment for three additional annual evaluations (phase II) began; 498 (62 %) of the 803 lead workers in the original cohort were re-enrolled (many had been laid-off in the economic crisis of the late 1990s and lost to follow-up) and 279 new participants were recruited. Inclusion criteria included occupational lead exposure and, for new participants, age $\geq$ 40 years in order to enrich the study with participants with increased risk for adverse kidney outcomes. There were no medical exclusionary criteria. At the end of this second enrollment phase (September 24, 2005), 778 current and former lead workers had completed the fourth evaluation. In order to optimize study data for both cross-sectional and longitudinal analyses despite funding constraints, a urine metals panel, including thallium, antimony and cadmium, was measured in fourth evaluation samples in the 712 workers who came to both the fourth and fifth evaluations. Workers from a primary smelter (n=28) who were enrolled in the second recruitment phase were excluded from this analysis due to their potentially wider range of occupational metal exposures. Thus, cross-sectional analysis of fourth evaluation data in the remaining 684 workers is the focus of the current analysis.

Data Collection

As previously described [70], data collection and biologic specimens included a standardized, interviewer-administered questionnaire; blood pressure measured with the IntelliSense™ blood

pressure monitor (Model HEM-907; Omron; Vernon Hills, IL); height and weight measurements; a blood specimen (for serum creatinine, cystatin C and blood lead); four-hour urine collection (for thallium, antimony, cadmium and creatinine levels); a spot urine sample(for NAG and creatinine) collected just before beginning the four-hour urine collection; and tibia lead.

Metals Exposure Assessment

Urine specimens were analyzed for metals in the Trace Elements section of the Laboratory of Inorganic and Nuclear Chemistry at the New York State (NYS) Department of Health's Wadsworth Center (Albany, NY, USA) which is the principal reference laboratory for the measurement of trace metals in urine in NYS. A multi-element method based on inductively coupled plasma mass spectrometry (ICP-MS) was used [72]. As previously described [70, 72], the method has been validated against the National Institute of Standards and Technology (NIST) Standard Reference Materials 2670a Toxic Elements in Urine, as well as secondary reference materials from a number of External Quality Assessment Schemes in which the lab participates successfully.

Urine specimens for trace metals analysis were collected and stored at -80°C in 5-mL Nalgene Cryogenic polypropylene tubes. Urine concentrations of thallium (m/z=205), antimony (m/z=121), and cadmium (m/z=114) were measured in standard mode using an Elan DRC II inductively coupled plasma mass spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT) equipped with dynamic reaction cell (DRC-ICP-MS) technology. As previously described [70, 72], 500 µL of urine was diluted 1+19 with 2% (v/v) HNO_3 (Veritas double-distilled; GFS Chemicals, Powell, OH), 0.005% Triton X-100 as a surfactant (Sigma-Aldrich, St. Louis, MO), 1 mg/L gold and 10 µg/L gallium, rhodium, yttrium, and iridium (Spex Certiprep, Inc., Metuchen, NJ) as internal standards. Multi-element calibration standards were prepared by serial dilution of NIST-traceable stock solution (High Purity Standards, Charleston, SC) using a six point calibration curve for each element. Base human urine pools were used to matrix-match the calibration standards. Samples were prepared under conditions (Clean Room and Class IIB

Biosafety Cabinet) certified as Class 100 or better to minimize the potential for contamination.

Quality control (QC) during the course of the study included analysis of urine-based internal quality control (IQC) materials before, during and after every analytical run. The mean coefficients of variation (CV) of the IQC samples from 18 days over the 5 month period in which the samples were assayed were 10% at 0.81 μg/L (n=74), 9.1% at 2.8 μg/L (n=74), 5.5% at 4.5 μg/L (n=62) for antimony and 8.5% at 0.25 μg/L (n=74), 8.9% at 0.70 μg/L (n=74), 5.3% at 0.95 μg/L (n=62) for thallium. One thallium value was below the method detection limit (MDL) of 0.02 μg/L; median (range) CV was 3.2 % (0.1-17.9) from 60 (8.8%) duplicate analyses (e.g., inter-assay CV). Of the antimony results, 313 (45.8%) were below the MDL of 0.2 μg/L; median (range) CV was 5.5 % (0.1-73.4 [of 9 CVs that were > 20%, 8 were from samples that were below the MDL]) from 60 duplicate analyses. Details of cadmium QC and correction for potential polyatomic interference from molybdenum were as previously published [70]. None of the results for cadmium were below the MDL of 0.02 μg/L; median (range) CV was 2.6% (0.2-19.1) based on 60 duplicate analyses.

Blood lead was measured with a Hitachi 8100 Zeeman background-corrected atomic absorption spectrophotometer [73] (Hitachi Ltd. Instruments, Tokyo, Japan) at the Institute of Industrial Medicine (now the Institute of Environmental & Occupational Medicine), a certified reference laboratory for lead in South Korea. Tibia lead levels were assessed via a 30-minute measurement of the left mid-tibia diaphysis using ^{109}Cd in a back-scatter geometry to fluoresce the K-shell X-rays of lead. The lead X-rays were recorded with a radiation detector and then quantified and compared to calibration data to estimate the concentration of lead in bone [74-76].

Kidney Outcome Assessment

Serum and urine creatinine were measured via a Dimension® clinical chemistry system using a Flex reagent cartridge in a modified kinetic Jaffe assay (model RxL; Dade Behring, Glasgow, DE, USA). Serum cystatin C was measured using an automated Dade Behring nephelometry assay on a Dimension Vista Lab System (Siemens Healthcare Diagnostics, Deerfield, IL, USA).

Urine NAG concentration was determined using a colorimetric assay (PPR Diagnostics Ltd, London, UK). For QC purposes, the original serum creatinine and cystatin C results were ordered by concentration and five percent of each was selected sequentially for duplication. Median inter-day CV for serum creatinine and cystatin C and urinary NAG samples run in duplicate were all < 10%.

Calculated kidney outcomes included calculated creatinine clearance [77]; measured creatinine clearance ([urinary creatinine in mg/dL × urine volume in mL]/serum creatinine in mg/dL / collection time in minutes); and the Modification of Diet in Renal Disease (MDRD) creatinine-based eGFR:

- $186.3 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times 0.742$ (if female) [15, 54].

Three cystatin-C-based equations were also used to estimate GFR [63]:

- single variable CYSeGFRs = $76.7 \times \text{serum cystatin C}^{-1.19}$
- multivariable CYSeGFRm = $127.7 \times \text{serum cystatin C}^{-1.17} \times \text{age}^{-0.13} \times 0.91$ if female
- Combined cystatin C/creatinine eGFRc = $177.6 \times \text{serum creatinine}^{-0.65} \times \text{serum cystatin C}^{-0.57} \times \text{age}^{-0.20} \times 0.82$ if female

Statistical Analysis

The goals of this analysis were to: 1) evaluate the respective associations between urine antimony and thallium levels and kidney outcomes (MDRD eGFR, measured and calculated creatinine clearances, serum creatinine, cystatin C, three cystatin-C-based eGFRs, and NAG) in current and former lead workers, while controlling for covariates; 2) to evaluate the effects of adjustment with the other urine metals on those associations (e.g., urine cadmium and antimony on models with thallium), also controlling for covariates, and; 3) to evaluate associations by dichotomized eGFR categories. Statistical analysis was completed using statistical software of the StataCorp LP (College Station, TX) [78].

Initially, variable distributions were examined. Both urine antimony and thallium, with and without adjustment for urine creatinine (μg/g creatinine) were right skewed and thus *ln*-transformed to minimize influential outliers. The NAG distribution also exhibited a non–normal distribution and was *ln*-transformed; the efficacy of this transformation was confirmed by examination of normal probability plots (Appendix Figures 3-1 (a-j)) and the final regression model residuals. In linear regression models, urine antimony and thallium dose were evaluated as a covariate in two ways: via the traditional approach in which the metal concentration is adjusted for urine dilution by dividing by urine creatinine; and via a more recent approach in which the urine metal and creatinine are both included as separate covariates in the model [79]. Antimony was initially analyzed using all data reported from the laboratory; subsequently it was determined that 45.8% of values were below the method detection limit. All analyses including antimony were then rerun with values below the method detection limit replaced by method detection limit/square root of 2 [80]. Results of this sensitivity analysis were consistent; hence results using actual values are reported.

Covariate selection utilized *a priori* variables (age, sex, and BMI [weight in kilograms divided by the square of height in meters]) in modeling that initially included urine antimony or thallium with other biologically relevant variables in separate models. Variables were retained in the final model if they substantially changed either the urine antimony or thallium regression coefficient or the explanatory value (r^2) of the model for any of the kidney outcomes, or were statistically significant, or were relevant based on *a priori* knowledge or hypotheses inherent to this study (e.g. blood and tibia lead; *ln*-urine cadmium). Additional covariates considered for inclusion using this approach were diabetes and hypertension (both based on participant report of physician diagnosis or medication use); regular analgesic use (based on questionnaire data on medication usage); self-reported work status (current vs. former lead worker); study status (phase I vs. II study participant), systolic and diastolic blood pressure (average of three measures); tobacco use (smoking status: never, former, current); smoking dose [(cigarettes per day × years of

smoking) in quartiles for current smokers and dichotomized for former smokers; alcohol consumption (never, former, current)]; education (< middle school graduate, < high school graduate, high school graduate, > high school graduate) and annual income (≤ 10, 10-20, 20-30, 30-40, and > 40 million won). Blood and tibia lead and urine cadmium were added to final models after all other covariates were selected. Job duration (years) was also added to the final antimony models because urine antimony is considered a recent occupational exposure measure in study participants employed in lead battery manufacturing facilities, and job duration may therefore approximate duration of antimony exposure.

Associations between each study metal were also examined in models stratified at the median of the respective outcome measure (MDRD eGFR, and CYSeGFRm) in order to assess the potential for reverse causality (i.e., whether associations were present only in participants with worse kidney function). Models were evaluated for linear regression assumptions and the presence of outlying points using augmented component-plus-residual plots and added-variable plots [81, 82]. Models were repeated without outliers when applicable. Models were also assessed for collinearity via examination of variance inflation factors.

Results

Selected Demographics, Exposure, and Health Outcome Measures

Information on demographics, metal dose biomarkers, kidney outcomes, and selected covariates is presented in Table 3-1 for all 684 lead workers and, separately, by current and former lead worker employment status. Males and current lead workers comprised 78.2 % (n=535) and 65.8% (n=450), respectively, of the population. Median urine thallium and antimony levels were 0.34 and 0.36 μg/g creatinine, respectively. Median antimony levels were substantially higher in current than former workers (0.87 and 0.1 μg/g creatinine, respectively), consistent with current occupational exposure. Antimony and thallium levels by plant type and worker status are shown in Appendix Tables 3-1 and 3-2. Mean values of the glomerular filtration measures were within

normal limits.

Exposure biomarkers were generally correlated (Table 3-2). Higher urine cadmium levels in former workers but higher blood lead and urine antimony levels in current workers were responsible for the lack of correlation between some of these metals. For example, antimony was positively correlated with cadmium in separate current and former worker groups (r=0.15, 0.16, respectively, $p<0.05$ for both) but not in all workers (r= -0.06, p=0.49). Urine thallium and cadmium were correlated with blood and tibia lead in current, but not former workers. Kidney outcomes and urine creatinine were also correlated (Table 3-3). Correlations were generally higher for measures based on the same biomarker (e.g., serum creatinine-based measures). Urine creatinine was not correlated with any cystatin C variables.

Associations of Urine Metals with Kidney Outcomes

In all lead workers, after adjustment for age, sex, BMI, current vs. former lead worker status, phase I vs. II study entry, annual income, education, alcohol consumption, smoking dose, diastolic blood pressure, blood lead, tibia lead, and, in antimony models only, lead job duration, higher urine concentrations of antimony and thallium, in separate models, were significantly associated with higher NAG, a direction consistent with nephrotoxicity (Table 3-3, Models 1 and 2, respectively). *Ln*-urine thallium was associated with 7 of the 8 serum creatinine and cystatin-C-based glomerular filtration measures; however, the directions were opposite that expected in traditional nephrotoxicity. *Ln*-urine antimony was only associated with serum creatinine-based glomerular filtration measures (serum creatinine and MDRD eGFR; a borderline significant association ($p < 0.1$) with calculated creatinine clearance was also observed). As with *ln*-urine thallium, the directions were opposite that expected in traditional nephrotoxicity. Neither metal was associated with measured creatinine clearance.

The direction of significant associations in fully adjusted models was the same in *a priori* models that adjusted only for age, sex, BMI and ln urine creatinine (Appendix Table 3-3).

Adjustment for blood and tibia lead attenuated associations between *ln*-urine thallium and kidney outcomes (although they remained highly significant) but slightly increased the beta coefficients of the associations between *ln*-urine antimony and the creatinine-based glomerular filtration measures (data without adjustment for lead dose not shown).

Multiple Metal Associations

Associations with each urine metal were evaluated following additional adjustment for the other metals, e.g., antimony further adjusted for thallium (Table 3-4, Model 3) and for thallium and cadmium (Table 3-4, Model 4). Previously significant associations with creatinine-based filtration outcomes were attenuated, however thallium associations remained significant and in the positive (unexpected) direction. With the exception of the combined creatinine- and cystatin-C-based eGFR, beta coefficients for *ln*-urine thallium associations with cystatin-C-based filtration measures became stronger with additional adjustment. Results were consistent when the metal concentrations were entered as μg/g creatinine (Appendix Table 3-4). The impact of cadmium adjustment on each metal is shown in Appendix Table 3-5.

Adjustment for metals that were strongly associated with kidney outcomes also had a large impact on urine creatinine associations. An example is the increased significance of urine creatinine in Models 2-4 for MDRD eGFR and CYSeGFRm compared to Model 1 (Table 3-4).

Sensitivity Analyses

In order to determine if the observed thallium and cadmium attenuation of antimony associations was related to measurement uncertainty (45.8% of antimony values were < MDL), sensitivity analyses in which values below the MDL were replaced by MDL/square root of 2 were conducted in models of MDRD eGFR. Similar evidence of attenuation of associations in combined metal models was observed (data not shown). Models were also run only in those participants with antimony values > MDL; attenuation, although observed, was less than when lower concentrations were included (data not shown).

To determine if the unexpected direction of the thallium and antimony associations was

related to different groups within our population, *a priori* models of MDRD eGFR stratified by worker status (current versus former) were examined. The direction of the associations was consistent with results in the combined population (data not shown). Similarly, consistent directions were observed in a priori models of MDRD eGFR stratified by participant sex (data not shown).

In models stratified by median kidney outcome, to assess the potential for reverse causality (Table 3-5), higher *ln*-urine thallium was associated only in the group of lead workers with worse kidney function (although in this occupational population, most kidney function values in this group were still in the normal range). In contrast, *ln*-urine antimony was positively associated only with MDRD eGFR and in the group with levels above the median. Similar results were observed in *a priori* models (data not shown).

In order to determine whether the thallium associations could be due to lead-related hyperfiltration, fully adjusted models were examined in 234 former lead workers whose blood lead levels were negatively associated with MDRD eGFR (β [95% CI] = -0.5 [-0.8, -0.1]), a pattern consistent with traditional lead-related nephrotoxicity. However, urine thallium remained positively associated with MDRD eGFR in these workers as well (β [95% CI] = 7.0 [1.9, 12.1]).

Discussion

We compared associations of *ln* -transformed urine antimony and thallium concentrations with a range of kidney outcomes, including eight serum creatinine and cystatin-C-based glomerular filtration measures and NAG, a proximal tubular early biological effect marker, to determine the impact of co-exposure to these metals on kidney function in lead exposed workers. Two key findings were observed. First, contrary to expectation, higher *ln*-urine thallium levels were significantly associated with higher levels of both serum creatinine-based and cystatin-C-based glomerular filtration measures (Table 3-4). These associations, which are in the opposite direction of those traditionally reported in nephrotoxicity, remained significant even after adjustment for

ln-urine cadmium and antimony and lead dose. Measured creatinine clearance was an exception; no significant associations with *ln*-urine antimony or thallium were observed. In subgroup analyses stratified by median kidney outcome, *ln*-urine thallium associations were limited to participants in the lower kidney function group (Table 3-5). Second, the analysis of multiple metals revealed evidence of attenuation. Antimony associations with kidney outcomes, only present with creatinine-based outcomes but in the same unexpected direction, became non-significant after thallium and cadmium adjustment (Table 3-4). Similarly, associations of NAG with both antimony and thallium were attenuated by cadmium adjustment.

Thallium and antimony are widespread in the environment. Exposure sources in the general population include industrial releases, from smelting of other metals and coal combustion, and diet due to uptake of metals from soil [67, 83, 84]. Elevated levels of thallium have been reported in trout from Lake Michigan [32]. In contrast to cadmium, urine levels of antimony and thallium reflect more current exogenous exposure [67]. Thallium is concentrated in the kidneys; the half-life in rats is approximately three days; data in humans are extremely limited but the half-life appears to be as long as 30 days [83]. A renal excretion half-life of four days was reported in lead battery production workers although a longer half-life may be possible based on reports of elevated urine levels in treated patients and deposits in lungs of smelter workers.[84] In our population, median urine thallium levels were similar between current and former lead workers (0.40 and 0.37 μg/g creatinine, respectively), however, urine antimony levels were higher in current workers (0.87 vs 0.10 μg/g creatinine in former workers), likely from occupational exposure to antimony used as an alloy in lead storage batteries. In comparison, median and 95th percentile urine levels in adults who participated in the 2003-2004 National Health and Nutrition Examination Survey (NHANES) were 0.15 and 0.33 μg/g creatinine, respectively, for thallium and 0.08 and 0.28 μg/g creatinine, respectively, for antimony [67].

Nephrotoxicity is a side effect of pentavalent antimony use in the treatment of parasitic diseases, such as leishmaniasis [85]. Increased serum creatinine, blood urea nitrogen and

proteinuria have been observed in animal models following 30 days of intramuscular injections with pentavalent antimony compounds [86]. Cortical tubular necrosis and decreased creatinine clearance have also been reported [85]. Interestingly, consistent with our results before adjustment for the other metals, decreased serum creatinine was observed in a dose-related manner in rats exposed to trivalent antimony salt via drinking water over a 90-day study period [87]. Route of administration may be relevant in animal data; nephrotoxicity from antimony potassium tartrate administered intraperitoneally but not via drinking water has been reported [88]. Tubular changes have also been observed in rabbits after 5 day inhalational exposures to antimony trisulfide [89]. A recent analysis of NHANES data identified antimony as a potential risk factor for peripheral arterial disease [90] and for cardiovascular and cerebrovascular disease [91].

Acute kidney injury has been reported in thallium poisoning [35, 36, 92]. Increased serum creatinine and proteinuria and decreased GFR have been observed with acute exposure in animal studies [39, 40, 93]. Pathologic changes have been reported throughout the renal tubule following acute and subacute thallium exposure in animal studies [37-39, 92, 94]. Decreased reabsorption in the proximal tubules, even without visible microscopic pathology, has also been observed [40]. We did not identify any comparable publications on associations between antimony or thallium and the kidney outcomes used in this analysis. Thallium was not associated with vascular outcomes in the NHANES analyses cited above for antimony [90, 91].

The data herein allow additional consideration of the previously published hypotheses [70, 95] generated from observed associations between *ln*-urine cadmium and kidney outcomes that, similar to thallium, were in the opposite direction traditionally expected in nephrotoxicity. In the current analysis, we modeled traditional serum creatinine-based kidney outcomes and measured creatinine clearance in a four- hour urine collection. We also included the kidney proximal tubule biomarker, NAG, as well as kidney outcomes based on serum cystatin C, a cysteine protease inhibitor that is secreted by all nucleated cells [62], thus avoiding confounding

with serum creatinine related to its metabolism from muscle. Since creatinine and cystatin C are correlated measures of kidney function, associations present with both creatinine and cystatin C-based eGFR suggest a kidney function, rather than an individual biomarker effect. Thus, associations between higher *ln*-urine thallium and higher levels of both serum creatinine- and cystatin-C-based glomerular filtration measures are consistent with a kidney function effect such as reverse causality or thallium-related hyperfiltration. Reverse causality implies that lower urine thallium levels reflect less kidney excretion in chronic kidney disease. In contrast, thallium-related hyperfiltration implies an effect of the metal on kidney function. Hyperfiltration is a longitudinal process reported in humans with diabetes, hypertension, and obesity [96] and lead-exposed rodents, [97] in which an initial increase in glomerular filtration rate is followed by a subsequent decline indicative of chronic kidney disease. Given that the thallium associations are confined to participants with filtration measures consistent with worse function, reverse causality seems more applicable than hyperfiltration. However neither hypothesis is as relevant for the antimony and cadmium associations observed with serum creatinine- but not cystatin-C-based filtration measures. As previously published, a statistical association related to the use of creatinine in both exposure and outcome metrics is another consideration for these findings [98]. The striking change in urine creatinine associations when a significant metal is in the model, as evidenced by Models 1 and 2 (Table 3-4) lends support for this hypothesis. However, given the differences in associations between metals, unique metal-specific mechanisms, such as metal-protein binding affecting excretion [7] must also be considered. Adding to the complexity of interpreting these findings is a recent publication in which lower levels of urinary bisphenol A were observed in NHANES participants with lower MDRD eGFR levels but not using a different creatinine-based eGFR measure [99].

We were able to exclude several potential hypotheses. Thallium associations cannot be attributed to lead-related hyperfiltration in this analysis since we adjusted for lead dose; moreover, urine thallium was positively associated with eGFR even in former workers in whom

lead-related hyperfiltration was not apparent. We were also able to exclude metal collinearity by observing consistent association directions in simpler *a priori* models and evaluating variance inflation factors in fully adjusted models. As an additional check on our models, BMI, a traditional CKD risk factor, was significantly and negatively associated (i.e., direction consistent with nephrotoxicity) with MDRD eGFR.

Novel results were also observed in the multiple metals analysis. Although common in environmental exposure, analysis of mixtures is infrequently evaluated in research. Co-exposures to metals with common target organs may have additive or multiplicative effects. A recent analysis of 1999-2006 NHANES data found that increased blood cadmium and lead levels were independently associated with the increased prevalence(s) of albuminuria and reduced eGFR [100]. Furthermore, odds of having both outcomes were four-fold higher in participants in the highest quartiles of both metals compared to those in the lowest. However, in the current analysis, attenuation was observed. In particular, NAG is routinely used in nephrotoxicant research. If we had limited our analysis to associations between antimony and thallium in separate models of NAG, we would have concluded that the results supported nephrotoxicity from both metals at low environmental levels of exposure. Instead, our multiple metal analysis allowed us to identify cadmium as the primary toxicant for NAG. The antimony attenuation observed in our analysis may be related to measurement uncertainty, given the large proportion of samples below the MDL, or to population differences due to higher exposure in the lead battery workers.

In conclusion, we report two novel and important findings in these data. First, urine thallium levels were significantly associated with both serum creatinine and cystatin-C-based glomerular filtration measures in the direction opposite to that traditionally reported with nephrotoxicity. Second, the analysis of multiple metals revealed attenuation of observed associations by other metals. These results suggest that interpretation of low-level urine metal values may be more complex than previously appreciated. Furthermore, single metal exposure analysis approaches may increase the potential for inaccurate risk conclusions. Additional

research is needed to determine the mechanism(s) for associations between higher urine metal concentrations and higher glomerular filtration measures.

Table 3-1. Selected demographic, exposure, and health outcome measures in current and former lead workers

	All Participants (n = 684)			Current workers (n= 450)		Former workers (n= 234)	
Characteristic	N (%)			N (%)		N (%)	
Male	535 (78.2)			421 (93.6)		114 (48.7)	
Diabetes	25 (3.6)			10 (2.2)		15 (6.4)	
Hypertension	84 (12.3)			45 (10.0)		39 (16.7)	
Current smokers	294 (43.0)			232 (51.6)		62 (26.5)	
	Median	Mean (SD)	Range	Median	Mean (SD)	Median	Mean (SD)
Age, years	46.5	47.6 (8.0)	24.1 - 71.3	44.7	45.2 (6.2)	54	52.0 (9.0)
BMI, kg/m^2	24.1	24.2 (2.9)	15.6 - 33.3	23.7	23.8 (2.7)	24.9	24.9 (3.1)
Systolic blood pressure, mm Hg	121.5	123.6 (15.7)	90.5 - 214.5	121.5	124.0 (15.4)	120.5	122.9 (16.3)
Diastolic blood pressure, mm Hg	74.5	75.1 (12.1)	46.0 - 148.0	74.5	75.9 (12.1)	73.0	73.6 (12.1)
Lead job duration, years	13.4	13.1 (7.2)	0.2 - 37.4	15.9	14.5 (6.5)	8.3	10.5 (7.7)
Antimony, µg/g creatinine	0.36	2.4 (7.5)	0.02 - 75.8	0.77	3.6 (9.0)	0.10	0.25 (0.48)
Thallium, µg/g creatinine	0.39	0.44 (0.23)	0.07 - 1.60	0.40	0.45 (0.23)	0.37	0.42 (0.24)
Cadmium, µg/g creatinine*	0.83	1.0 (0.62)	0.17 - 4.09	0.75	0.84 (0.43)	1.08	1.31 (0.79)
Blood lead, µg/dL	21.5	23.2 (14.3)	1.9 - 74.4	27.4	29.1 (13.0)	8.8	11.8 (8.8)
Tibia lead, µg Pb/g bone mineral**	20	27.1 (29.3)	-12 - 231	20	27.0 (26.9)	19	27.4 (33.5)
Serum creatinine, mg/dL	0.87	0.87 (0.15)	0.42 -1.53	0.89	0.88 (0.14)	0.81	0.83 (0.17)
MDRD eGFR, mL/min/1.73 m2	95.8	97.7 (19.4)	23.6 - 189.7	97.9	100.2 (18.1)	90.7	92.9 (20.8)
Calculated Creatinine Clearance, ml/min	93.9	95.4 (22.2)	30.5-209.9	97.3	98.9 (20.1)	86.7	88.7 (24.4)
Measured creat. clearance, mL/min	110.4	110.8 (30.9)	9.9 - 222.9	116.3	116.4 (30.8)	97.8	99.9 (28.3)
Serum cystatin C, mg/L	0.72	0.73 (0.12)	0.50 - 2.35	0.71	0.72 (0.11)	0.74	0.76 (0.13)
CYSeGFRm, ml/min/1.73 m2	112.7	112.0 (17.8)	28.1 - 186.3	116.4	116.6 (15.5)	102.3	103.1 (18.6)
CYSeGFRs, mL/min/1.73 m2	113.8	113.7 (16.9)	27.7 – 176.3	115.4	116.2 (15.4)	109.0	108.8 (18.4)
Combined eGFRc, mL/min/1.73 m2	106.0	106.3 (17.7)	24.3 – 174.5	109.3	109.8 (15.7)	98.0	99.6 (19.3)
NAG µmol/h/g creatinine	318.1	386.3 (282.1)	46.1 - 3778.5	289.1	345.7 (230.3)	399.4	464.2 (348.9)

*molybdenum corrected; **n=678

Table 3-2. Spearman's correlation coefficients for metal dose variables in 684 current and former lead workers

All Workers

	Antimony[a]	Thallium	Cadmium	Blood Lead	Tibia
Thallium, μg/g creatinine	0.23#				
Cadmium, μg/g creatinine	-0.06	0.21#			
Blood Lead, μg/dL	0.51#	0.17#	-0.12**		
Tibia Lead, μg/g bone	0.19#	0.16#	0.08*	0.47#	
Job Duration, years	0.39#	0.11**	-0.11**	0.32#	0.41#

Current Workers

	Antimony[a]	Thallium	Cadmium	Blood Lead	Tibia
Thallium, μg/g creatinine	0.19#				
Cadmium, μg/g creatinine	0.15**	0.22#			
Blood Lead, μg/dL	0.19#	0.19#	0.17#		
Tibia Lead, μg/g bone	0.18#	0.22#	0.13**	0.57#	
Job Duration, years	0.17#	0.06	0.004	-0.06	0.36#

Former Workers

	Antimony[a]	Thallium	Cadmium	Blood Lead	Tibia
Thallium, μg/g creatinine	0.32#				
Cadmium, μg/g creatinine	0.16*	0.30#			
Blood Lead, μg/dL	0.44#	0.05	0.02		
Tibia Lead, μg/g bone	0.28#	0.04	0.06	0.61#	
Job Duration, years	0.43#	0.14*	0.02	0.60#	0.51#

*p-value < 0.05; **p-value < 0.01; #p-value < 0.001; [a]Antimony (μg/g creatinine)

Table 3-3. Spearman's correlation coefficients for urine creatinine and kidney outcome measures in 684 lead workers

Kidney Outcome	Calc. Creat. Clearance	MDRD eGFR	Meas. Creat. Clearance	Cystatin C	CYS eGFRs	CYS eGFRm	Combined eGFRc	NAG	Urine Creatinine
Serum Creatinine, mg/dL	-0.38#	-0.72#	-0.17#	0.37#	-0.37#	-0.18#	-0.58#	-0.22#	0.14#
Calculated Creatinine Clearance, ml/min		0.69#	0.62#	-0.37#	0.37#	0.47#	0.69#	-0.11**	0.15#
MDRD eGFR, ml/min/1.73 m^2			0.49#	-0.48#	0.48#	0.51#	0.91#	0.001	0.04
Measured Creatinine Clearance, mL/min				-0.28#	0.28#	0.38#	0.51#	-0.20#	0.33#
Serum Cystatin C, mg/L					-1.0#	-0.93#	-0.76*	0.06	0.07
Single variable CysCeGFRs, mL/min/1.73 m^2						0.93#	0.76#	-0.06	-0.07
Multi-variable CysCeGFRm, mL/min/1.73 m^2							0.80#	-0.18#	0.01
Combined eGFRc, mL/min/1.73 m^2								-0.08*	0.03
Ln-NAG, µmoL/h/g creatinine									-0.12**

* p-value < 0.05; ** p-value < 0.01; # p-value < 0.001

Table 3-4. Associations between urine metals and kidney outcomes with and without cadmium adjustment in 684 lead workers

Kidney Outcome	Model 1 β coeff (95 % CI))	Model 2 β coeff (95 % CI))	Model 3 β coeff (95 % CI))	Model 4 β coeff (95 % CI))
MDRD eGFR, ml/min/1.73 m^2				
Ln-Urine Antimony μg/L	1.5 (0.5, 2.5)**		1.0 (-0.03, 1.9)	0.8 (-0.2, 1.7)
Ln- Urine Thallium, μg/L		6.8 (4.1, 9.6)#	6.4 (3.6, 9.2)#	5.2 (2.4, 8.0)#
Ln-Urine Cadmium, μg/L				6.7 (3.5, 9.9)#
Ln-Urine Creatinine, mg/dl	-2.0 (-4.4, 0.4)	-6.3 (-9.5, -3.0)#	-6.8 (-10.1, -3.5)#	-11.7 (-15.8, -7.7)#
Calculated Creatinine Clearance, mg/dL				
Ln-Urine Antimony μg/L	0.8 (-0.1, 1.6)		0.5 (-0.3, 1.4)	0.4 (-0.4, 1.3)
Ln- Urine Thallium, μg/L		4.0 (1.5, 6.5)**	3.8 (1.3, 6.2)**	3.0 (0.6, 5.4)*
Ln-Urine Cadmium, μg/L				3.5 (0.7, 6.3)*
Ln-Urine Creatinine, mg/dl	0.1 (-1.9, 2.2)	-2.5 (-5.4, 0.5)	-3.0 (-5.9, -0.1)*	-5.5 (-9.0, -1.9)**
Measured Creatinine Clearance, mL/min/1.73 m^2				
Ln-Urine Antimony μg/L	-0.6 (-2.1, 0.9)		-0.9 (-2.4, 0.6)	-0.7 (-2.2, 0.8)
Ln- Urine Thallium, μg/L		2.7 (-1.4, 6.8)	3.1 (-1.0, 7.3)	3.6 (-0.6, 7.8)
Ln-Urine Cadmium, μg/L				-3.3 (-8.1, 1.4)
Ln-Urine Creatinine, mg/dl	11.3 (7.8, 14.8)#	8.2 (3.4, 13.1)**	8.8 (3.8, 13.7)**	11.3 (5.2, 17.3)#

Serum Creatinine, mg/dL				
Ln-Urine Antimony µg/L	-0.010 (-0.018,-0.003)**		-0.006 (-0.013, 0.001)	-0.005 (-0.012, 0.003)
Ln- Urine Thallium, µg/L		-0.053 (-0.073,-0.033)#	-0.050 (-0.071, -0.030)#	-0.042 (-0.063, -0.021)#
Ln-Urine Cadmium, µg/L				-0.049 (-0.073, -0.026)#
Ln-Urine Creatinine, mg/dl	0.013 (-0.005, 0.031)	0.051 (0.027, 0.075)#	0.054 (0.030, 0.079)#	0.091 (0.061, 0.121)#
CYSeGFRm, mL/min/1.73 m^2				
Ln-Urine Antimony µg/L	-0.2 (-1.0, 0.7)		-0.5 (-1.3, 0.3)	-0.4 (-1.3, 0.4)
Ln-Urine Thallium µg/L		4.9 (2.6, 7.2)#	5.1 (2.8, 7.4)#	5.4 (3.1, 7.8)#
Ln- Urine Cadmium				-1.9 (-4.6, 0.7)
Ln-Urine Creatinine, mg/dl	-0.6 (-2.6, 1.3)	-5.2 (-7.8, -2.5)#	-4.9 (-7.6, -2.2)#	-3.5 (-6.8, -0.1)*
CYSeGFRs, mL/min/1.73 m^2				
Ln-Urine Antimony µg/L	-0.1 (-1.0, 0.7)		-0.5 (-1.3, 0.4)	-0.4 (-1.3, 0.4)
Ln-Urine Thallium µg/L		5.3 (2.9, 7.7)#	5.5 (3.1, 7.9)#	5.8 (3.4, 8.3)#
Ln- Urine Cadmium				-2.0 (-4.7, 0.8)
Ln-Urine Creatinine, mg/dl	-0.6 (-2.7, 1.4)	-5.5 (-8.3, -2.7)#	-5.2 (-8.1, -2.4)#	-3.8 (-7.2, -0.3)*

Combined eGFRc, mL/min/1.73 m^2				
Ln-Urine Antimony μg/L	0.9 (-0.01, 1.7)		0.4 (-0.5, 1.2)	0.3 (-0.5, 1.1)
Ln-Urine Thallium μg/L		6.3 (3.9, 8.6)#	6.1 (3.7, 8.5)#	5.6 (3.1, 8.0)#
Ln- Urine Cadmium				3.1 (0.4, 5.9)*
Ln-Urine Creatinine, mg/dl	-1.5 (-3.5, 0.6)	-6.3 (-9.1, -3.5)#	-6.5(-9.3, -3.7)#	-8.8 (-12.3,-5.3)#
Serum Cystatin C, mg/L				
Ln-Urine Antimony μg/L	0.0004 (-0.004, 0.005)		0.002 (-0.002, 0.007)	0.002 (-0.003, 0.006)
Ln-Urine Thallium μg/L		-0.032 (-0.045, -0.020)#	-0.033 (-0.046, -0.021)#	-0.036 (-0.048, -0.023)#
Ln- Urine Cadmium				0.015 (0.001, 0.030)*
Ln-Urine Creatinine, mg/dl	0.005 (-0.006, 0.015)	0.034 (0.019, 0.048)#	0.032 (0.018, 0.047) #	0.021 (0.003, 0.039)*
***Ln*-NAG, μmoL/h/g creatinine**				
Ln-Urine Antimony	0.033 (0.004, 0.063)*		0.029 (-0.0004, 0.058)	0.022 (-0.007, 0.052)
Ln-Urine Thallium μg/L		0.096 (0.015, 0.178)*	0.083 (0.001, 0.166)*	0.045 (-0.038, 0.128)
Ln- Urine Cadmium	-	-		0.225 (0.132, 0.319)#
Ln-Urine Creatinine, mg/dl	-0.101(-0.171, -0.031)**	-0.154 (-0.251, -0.057)**	-0.171(-0.269, -0.073)**	-0.337 (-0.456, -0.218)**

Models also adjusted for age, gender, BMI, employment status (current vs. former lead worker), enrollee status (phase I vs. II study entry), annual income (10, 10-20, 20-30, 30-40, and > 40 million won), education (< middle school graduate, < high school graduate, high school graduate, > high school), alcohol consumption (never, former, current), smoking dose [(cigarettes per day × years of smoking) in quartiles for current smokers and ex-smoker status], diastolic blood pressure, blood lead, and tibia lead. Model 1 also adjusted for lead job duration

*p-value < 0.05; **p-value < 0.01; #p-value < 0.001

Table 3-5. Associations between urine metals and eGFR in models stratified at median kidney outcome (n =684)[a]

Kidney Function Measure	β coeff (95 % CI)	β coeff (95 % CI)
MDRD eGFR, ml/min/1.73 m^2	**< 95.9 ml/min/1.73 m^2**	**≥ 95.9 ml/min/1.73 m^2**
Ln-urine antimony, µg/L	-0.3 (-1.0, 0.5)	1.2 (0.1, 2.2)*
Ln-urine thallium, µg/L	2.4 (0.6, 4.2)*	-0.1 (-3.8, 3.7)
Ln-urine cadmium, µg/L	3.0 (0.7, 5.4)*	5.2 (1.6, 8.8)**
Calculated Creatinine Clearance, ml/min	**< 93.9 ml/min**	**≥ 93.9 µg/dL**
Ln-urine antimony, µg/L	0.4 (-0.3, 1.1)	1.0 (-0.03, 2.1)
Ln-urine thallium, µg/L	2.6 (0.8, 4.4)**	-0.02 (-3.5, 3.5)
Ln-urine cadmium, µg/L	2.7 (0.5, 5.0)**	5.0 (1.3, 8.7)**
Serum Creatinine, µg/L	**< 0.87 µg/dL**	**≥ 0.87 µg/dL**
Ln-urine antimony, µg/L	-0.005 (-0.012, 0.002)	0.002 (-0.006, 0.009)
Ln-urine thallium, µg/L	-0.017 (-0.039, 0.005)	-0.024 (-0.044, -0.004)*
Ln-urine cadmium, µg/L	-0.040 (-0.061, -0.019)#	-0.030 (-0.057, -0.003)*
CYSeGFRm, ml/min/1.73 m^2	**< 112.7 ml/min/1.73 m^2**	**≥ 112.7 ml/min/1.73 m^2**
Ln-urine antimony, µg/L	-0.4 (-1.3, 0.5)	-0.4 (-1.1, 0.4)
Ln-urine thallium, µg/L	4.5 (2.6, 6.5)#	1.0 (-1.9, 3.9)
Ln-urine cadmium, µg/L	-2.9 (-5.3, -0.5)*	0.3 (-2.5, 3.2)

[a] Models also adjusted for age, sex, BMI, ln-urine creatinine, employment status (current vs. former lead worker), smoking dose (cigarettes per day × years of smoking) in quartiles for current smokers and ex-smoker status, alcohol consumption (never, former, current); education (< middle school graduate, < high school graduate, high school graduate, > high school), annual income (≤ 10, 10-20, 20-30, 30-40, and > 40 million won), enrollee status (phase I vs. II study entry), diastolic blood pressure, and blood and tibia lead.

*p-value < 0.05; **p-value < 0.01; #p-value < 0.001.

Chapter 4: ASSOCIATIONS OF ENVIRONMENTAL URANIUM EXPOSURE WITH KIDNEY OUTCOMES IN LEAD WORKERS (MANUSCRIPT II)

Abstract

Background: Uranium is a ubiquitous metal that is nephrotoxic at acute high dose exposures. However, few epidemiologic studies have examined the impact of chronic environmental uranium exposures on kidney function.

Objectives: To evaluate associations of urine uranium concentrations with kidney outcomes in lead workers environmentally exposed to uranium.

Methods: Multiple linear regression was used to examine associations between *ln*-urine uranium with measured creatinine clearance, serum creatinine- and cystatin-C-based glomerular filtration measures, and N-acetyl-β-D-glucosaminidase (NAG).

Results: In 684 participants, median urine uranium levels were 0.07 μg/g creatinine and 0.02 μg total uranium excreted over the four hour urine collection period. These measures were highly correlated (r_s=0.95; $p < 0.001$). After adjustment for lead dose, urine thallium and cadmium, and kidney risk factors, higher *ln*-urine uranium was associated with lower measured creatinine clearance and higher NAG in models that used urine creatinine to adjust for urine dilution. In contrast, these associations were not present in the model that used total uranium excreted (μg) rather than urine creatinine to adjust for urine dilution.

Conclusions: Urine uranium levels were significantly associated with measured creatinine clearance and NAG only when urine creatinine was included in the regression model. This suggests that these results may be, in part, due to spurious biostatistical associations created by the use of urine creatinine in both exposure and outcome metrics. These findings support the need to consider non-creatinine based methods of adjustment for urine dilution as well as additional

non-urine based exposure measures (e.g. blood levels) in kidney research that uses creatinine-based outcomes.

Introduction

Identification of environmental nephrotoxicants is increasingly important as the world-wide prevalence of chronic kidney disease (CKD) grows.[101] Uranium is an important consideration in this regard. The US Nutrition and Health Examination Survey (NHANES), considered representative of the US general population, has consistently detected uranium in the urine of a majority of the study participants[102]. Exposure in the general population occurs mainly through ingestion of uranium on food grown in contaminated soil and in drinking water. Exposure to uranium via contaminated ground water is a global concern as elevated levels have been detected in a wide range of geographic areas such as eastern and southwest United States, Canada, Scandinavian countries, central Australia, India, and South Korea.[41, 43, 44] Increased exposure potential through consumption of unmonitored well water is particularly a concern in rural areas and less affluent countries where there are few regulated public drinking water systems. The main industrial use of uranium is as a power source for nuclear reactors,[43] a use which is expected to rise with increasing energy demand.[45] Therefore, exposure potential related to uranium mining activities and nuclear power plant emissions will be an important consideration into the future.

Despite the knowledge of widespread environmental exposure, epidemiologic research on associations between chronic low-level uranium exposure and kidney outcomes remains limited. Uranium is nephrotoxic at acute high dose exposures in humans and animals.[41, 103] Limited evidence suggests biomarkers of kidney dysfunction may be present in populations chronically exposed to relatively low levels of uranium.[41, 42, 103] Additionally, the nephrotoxic effects of exposure to multiple metals are relatively unknown. Populations co-exposed to uranium, lead and/or other nephrotoxic metals may be at increased risk for kidney damage. Thus the data to date indicate considerable potential for nephrotoxicity associated with environmental uranium

exposure and support the need for additional epidemiologic research. To address this knowledge gap, we performed a cross-sectional analysis of associations between urine uranium levels and kidney outcomes in 684 current and former lead workers in the Republic of Korea in whom cadmium, antimony, thallium, associations with kidney outcomes had also been analyzed [104] (see also Chapter 3).

Materials and Methods

Study Overview and Design

We performed a cross-sectional analysis of data from current and former inorganic lead workers who were voluntary participants in a longitudinal study established to look at health effects of occupational lead exposure. Data used for this analysis was obtained between April 2004 and September 2005 (phase II enrollment). All participants provided written, informed consent and the study protocol was approved by Institutional Review Boards at the SoonChunHyang University School of Medicine and the Johns Hopkins University Bloomberg School of Public Health.

Study Population

As previously described [12, 71, 104], participants in the first enrollment phase of the cohort study were recruited between 1997 and 1999 through medical surveillance programs at secondary lead smelters and plants that produced lead batteries, lead oxide, lead crystal, or radiators. The population is 100% Korean. Inclusion criteria were occupational exposure to lead and, for phase II enrollees, age $\geq$ 40 years in order to enrich the study with participants with increased risk for adverse kidney outcomes. There were no medical exclusionary criteria. In order to optimize study data for both cross-sectional and longitudinal analyses, a urine metals panel, including uranium, thallium, and cadmium, was measured in the fourth evaluation samples of the 712 workers who participated in both the fourth and fifth evaluations. Workers from a primary smelter (n=28) were excluded from this analysis due to their potential wider range of occupational metal exposures.

Thus, cross-sectional analysis of data in the remaining 684 workers was the focus of the current analysis.

Data Collection

As previously described [104], data collection and biologic specimens included a standardized, interviewer-administered questionnaire; blood pressure obtained with the IntelliSense™ blood pressure monitor (Model HEM-907; Omron; Vernon Hills, IL); height and weight; a blood sample (for serum creatinine, cystatin C and blood lead); a four-hour urine collection (for uranium, thallium, cadmium and creatinine levels); a spot urine sample collected just before beginning the four-hour urine collection (for NAG and creatinine); and tibia lead.

Metals Exposure Assessment

Urine specimens were analyzed for metals in the Trace Elements section of the Laboratory of Inorganic and Nuclear Chemistry at the New York State (NYS) Department of Health's Wadsworth Center (Albany, NY, USA), which is the principal reference laboratory for the measurement of trace metals in urine in NYS. A multi-element method based on inductively coupled plasma mass spectrometry (ICP-MS) was used [105]. As previously described [104], the method has been validated against the National Institute of Standards and Technology Standard Reference Materials 2670a Toxic Elements in Urine. The Laboratory also successfully participates in a number of External Quality Assessment Schemes.

The urine specimens were collected and frozen in containers that were pre-certified for low-level cadmium measurements and stored at -80°C in 5-mL Nalgene Cryogenic polypropylene tubes. Urine metal concentrations were measured using an Elan DRC II inductively coupled plasma mass spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT) equipped with dynamic reaction cell (DRC) technology. Briefly, 500 µL of urine was diluted 1+19 with 2% (v/v) HNO_3 (Veritas double-distilled; GFS Chemicals, Powell, OH), 0.005% Triton X-100 as a surfactant (Sigma-Aldrich, St. Louis, MO), 1 mg/L gold and 10 µg/L gallium, rhodium, yttrium, and iridium (Spex Certiprep, Inc., Mutuchen, NJ) as internal standards. Multi-element calibration

standards were prepared by serial dilution of National Institute of Science and Technology (NIST)-traceable stock solution (High Purity Standards, Charleston, SC) using a six point calibration curve for each element. Base human urine pools were used to matrix-match the calibration standards. Samples were prepared under conditions (Clean Room and Class IIB Biosafety Cabinet) certified as Class 100 or better to minimize the potential for contamination.

Quality control (QC) included analysis of urine-based internal quality control (IQC) materials before, during and after every analytical run. For uranium, the mean coefficient of variation of the IQC samples from 18 days over the 5 month period in which the samples were assayed was 19% at 0.03 μg/L (n=74), 13% at 0.06 μg/L (n=74), 10% at 0.09 μg/L (n=62).

Blood lead was measured with a Hitachi 8100 Zeeman background-corrected atomic absorption spectrophotometer[73] (Hitachi Ltd. Instruments, Tokyo, Japan) at the Institute of Industrial Medicine, a certified reference laboratory for lead in South Korea. Tibia lead levels were assessed via a 30-minute measurement of the left mid-tibia diaphysis using ^{109}Cd in a back-scatter geometry to fluoresce the K-shell X-rays of lead. The lead X-rays were recorded with a radiation detector and then quantified and compared to calibration data to estimate the concentration of lead in bone [74-76].

Kidney Outcome Assessment

Serum and urine creatinine levels were obtained by a Dimension® clinical chemistry system using a Flex reagent cartridge in a modified kinetic Jaffe assay (model RxL; Dade Behring, Glasgow, DE, USA). Serum cystatin C was measured via an automated Dade Behring nephelometry assay on a Dimension Vista Lab System (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The PPR NAG test kit was used to determine urine NAG concentrations (PPR Diagnostics Ltd, London, UK. QC results for serum creatinine and cystatin C, and NAG results were as described in Chapter 3.

Calculated kidney outcomes included:

- Measured creatinine clearance:

([urine creatinine in mg/dl × urine volume in ml]/serum creatinine in mg/dl) / collection time in minutes)

- The Modification of Diet in Renal Disease (MDRD) creatinine-based eGFR: [15, 106]:

  $186.3 * (\text{serum creatinine})^{-1.154} * (\text{age})^{-0.203} * 0.742 \text{ (if female)}$

- And a multi-variable cystatin-C-based (CYSeGFRm) equation: [63]

  $127.7 \times \text{serum cystatin } C^{-1.17} \times \text{age}^{-0.13} \times 0.91 \text{ if female}$

Statistical Analysis

The goals of the statistical analysis were to evaluate associations between urine uranium levels and kidney outcomes (MDRD eGFR, CYSeGFRm, and NAG) in current and former lead workers, while controlling for covariates; including blood and tibia lead and urine thallium and cadmium. Statistical analysis was completed using statistical software of the StataCorp LP (College Station, TX).[78]

Initially, variable distributions were examined. Urine uranium, thallium, and cadmium expressed with and without adjustment for urine creatinine (μg /g creatinine and μg/L, respectively) and as total μg excreted in the four-hour urine collection, were right skewed and subsequently *ln*-transformed to minimize influential outliers. The NAG data also exhibited a non–normal distribution and was *ln*-transformed. The efficacy of these transformations was confirmed by examination of standardized normal probability plots (Appendix Figures 4-1 (a-m) & 3-1 (e-j) and the final regression model residuals.

In linear regression models, urine uranium dose was evaluated using three different methods to adjust for urine dilution: the traditional approach in which the metal concentration is adjusted for urine dilution by dividing by urine creatinine; a more recent approach in which the urine metal and creatinine are included as separate covariates in the model[79] and; a non-creatinine based approach using the total amount of uranium excreted during the four-hour urine-collection.

Initial regression models included uranium and *a priori* variables (age, gender, and BMI [weight in kilograms divided by the square of height in meters]). Additional covariates considered for inclusion were diabetes and hypertension (both based on participant report of physician diagnosis or medication use); regular analgesic use (based on questionnaire data on medication usage); self-reported work status (current vs. former lead worker); lead job duration (years); study status (original vs. new enrollee), systolic and diastolic blood pressure (average of three measures); tobacco use (smoking status: never, former, current); smoking dose [(cigarettes per day x years of smoking) in quartiles for current smokers and dichotomized for former smokers; alcohol consumption (never, former, current)]; education (< middle school graduate, < high school graduate, high school graduate, > high school) and annual income (≤ 10, 10-20, 20-30, 30-40, and > 40 million won). Variables were retained in the final model if they substantially changed the uranium regression coefficient or the explanatory value (r^2) of the model for any of the kidney outcomes, were statistically significant ($p \leq 0.05$), or were relevant based on *a priori* knowledge or hypotheses inherent to this study. (e.g. *ln*-urine cadmium). Blood and tibia lead and urine thallium and cadmium were added to final models after all other covariates were selected.

Models were evaluated for linear regression assumptions and the presence of outlying points using augmented component-plus-residual plots and added-variable plots [81] and repeated without outliers when applicable. Models were also assessed for collinearity through examination of variance inflation factors.

Results

Information on demographics, uranium and other metal dose biomarkers, kidney outcomes, and selected covariates is presented in Table 4-1. Males comprised 78.2 % (n=535) of the population. Median urine uranium levels were 0.07 μg/g creatinine and 0.02 μg excreted in four hours. Median urine metal levels (μg/g creatinine and μg excreted) were 0.39 and 0.09 for thallium and 0.83 and 0.19 for cadmium, respectively.

Urine uranium, expressed as μg/g creatinine, was positively correlated with urine thallium in μg/g creatinine and negatively correlated with blood lead (r_s = 0.09 and -0.21, respectively, $p < 0.05$; Table 2). Total uranium excreted (μg) was positively correlated with total thallium excreted (μg), and negatively correlated with urine cadmium (μg/g creatinine), and blood lead (r_s= 0.12, -0.09, and -0.15, respectively, $p < 0.05$; Table 4-2). Creatinine adjusted urine metals were all strongly correlated with their respective total metal excreted concentrations ($r_s >$ 0.75; $p < 0.001$ for all; Table 4-2). As reported in Chapter 3, measured creatinine clearance was positively correlated with MDRD eGFR and negatively correlated with NAG (r_s = 0.49 and -0.20 respectively; $p<0.001$ for both). Urine creatinine, measured over the four-hour collection period, was correlated with measured creatinine clearance, spot urine creatinine (used for NAG adjustment), and NAG (r_s= 0.33, 0.48, and -0.12 respectively; $p<0.01$ for all), but was not associated with either eGFR equation.

Associations of Urine Uranium with Kidney Outcomes

Higher *ln*-urine uranium was associated with lower measured creatinine clearance and higher NAG after adjustment for age, sex, BMI, current vs. former lead worker status, phase I vs. II study entry, annual income, education, alcohol consumption, smoking dose, diastolic blood pressure, blood lead, tibia lead, and *ln*-urine concentrations of thallium, cadmium, in the two models that used urine creatinine to adjust for urine dilution (Table 4-3). In Model 1, *ln*-urine creatinine was entered as a separate co-variate, and in Model 2, uranium and the other urine metals were divided by urine creatinine and the resulting variable, in μg/g creatinine, was ln transformed and entered so that *ln*-urine creatinine was not entered as a separate co-variate. In contrast, when urine dilution adjustment was achieved by entering the metals as μg excreted in the four-hour urine collection, no association between total uranium excreted (μg) and measured creatinine clearance or NAG was observed (Table 4-3; Model 3). Uranium was not associated with either creatinine- or cystatin-C-based eGFR in any of the three models (Table 4-3).

Results in *a priori* models that adjusted for age, sex, BMI, and, in models with *ln*-urine uranium entered as μg/L, urine creatinine, were consistent. Similarly, as with the fully adjusted model, there were no significant associations with any of the kidney outcomes in the *a priori* total uranium excreted (μg) models (data not shown). These results were also consistent in fully adjusted models that did not include urine thallium or cadmium (Appendix Table 4-1).

Discussion

We examined associations of *ln*-transformed urine uranium concentrations with four kidney outcome measures (three filtration measures: measured creatinine clearance and serum creatinine- and cystatin-C-based glomerular filtration rates; and NAG, a proximal tubule marker) to determine the impact of exposure to uranium on kidney function in lead exposed workers. We employed three different methods of adjustment for urine dilution and compared consistency of results across the three respective linear regression models. Distinct differences in association by method of adjustment for dilution were observed. *Ln*-urine uranium was significantly associated with lower measured creatinine clearance and higher NAG in the two models that used urine creatinine to adjust for urine dilution; where one approach included urine creatinine as a separate covariate in the regression model, and the other approach included the more traditional creatinine adjusted metal (i.e. urine uranium expressed in μg/g creatinine) as a single variable. These findings are consistent with nephrotoxic effects. In contrast, associations were no longer evident in the third approach, which used total uranium excreted (μg) rather than urine creatinine to adjust for urine dilution.

Uranium is a ubiquitous heavy metal naturally found in rocks and soil. It is mined and used as an energy source as well as in a range of products such as glass tint, glazes, and military munitions. Environmental exposure to uranium occurs mainly through ingestion of ground water or food.[42, 46] Absorption of uranium in the GI tract is generally poor (< 3.2 % in humans) with the greatest absorption occurring in a fasting state or in more water soluble forms.[27, 47] Urine

is the primary route of excretion for absorbed uranium.[27, 42, 46] An estimated 66% of uranium that enters the human bloodstream is rapidly excreted within 24 hours and nearly 90% within a month. The remainder is primarily distributed to bone, and to a lesser extent, liver and kidneys.[47] The half-life in bone may range from months to years. Therefore, urine uranium is considered to be a recent exogenous dose measure with a small contribution from cumulative endogenous exposure [46]. Median and 95th percentile urine uranium levels in our population were 0.070 and 0.531 μg/g creatinine compared to 0.006 and 0.026 μg/g creatinine in 2005-2006 NHANES participants.

Animal studies using a variety of uranium compounds have indicated that uranium is nephrotoxic. Morphological abnormalities and dysfunction of the proximal tubule and glomerulus have been reported in acute and subacute studies [41, 42, 48, 107] with proximal tubule damage reported from chronic exposure.[41, 48, 108] Evidence of morphological glomerular and proximal tubule damage has also been found in several acute high dose human case reports, such as in industrial accidents.[42] Epidemiological studies have reported associations between uranium and adverse proximal tubule effects. Beta 2-microglobulin is the proximal tubule biomarker that has been most consistently associated with uranium exposure in humans.[41]

Epidemiological studies examining associations between uranium exposure and glomerular filtration measures are scarce and the results are inconsistent (Table 4-4). Findings range from no associations [109-113] to associations consistent with nephrotoxicity (increased serum creatinine[114] and increased serum beta-2-microglobulin[115]); to associations opposite that expected in the traditional nephrotoxicity pattern (lower serum creatinine[115-118] and higher creatinine clearance[115] or higher GFR[118]). In the studies in Table 4-4 in which NAG was included, no significant associations were observed, similar to our results with the total uranium excreted model.[110, 113, 117, 118]

In the data herein, increased *ln*-urine uranium was associated with decreased measured creatinine clearance and increased NAG, but only if urine dilution adjustment involved the use of

urine creatinine and not if total uranium excreted (μg) was used as the exposure metric. This inconsistency is all the more surprising since creatinine adjusted urine uranium (μg/g creatinine) was strongly correlated (r_s=0.95; $p < 0.001$) with total uranium excreted (μg) and thus consistent results using these exposure metrics would be expected. This suggests that the significant associations observed may be biostatistical aberrations due to the use of urine creatinine in both exposure and outcome metrics. Urine uranium is directly divided by the four-hour urine creatinine when entered as μg/g creatinine; the equivalent effect is achieved when both variables are entered separately into the model. The same urine creatinine value was also used to calculate measured creatinine clearance:

([urine creatinine in mg/dl × urine volume in ml]/serum creatinine in mg/dl) /collection time in minutes

Additionally, NAG was directly divided by the spot urine creatinine (μg/g creatinine), which was correlated with the four-hour urine creatinine (r_s = 0.48, $p<0.001$).

To our knowledge, there are no published studies that have compared results among creatinine-based methods of adjustment for urine dilution and total uranium excreted (μg). However, Kurrtio et al, (2006) found no association between non-creatinine adjusted urine uranium (μg/L) and NAG, serum cystatin-C, or measured creatinine clearance, noting that results were similar when using creatinine-corrected urinary uranium and also using uranium exposure measures that did not require adjustment for dilution (e.g. uranium in hair and toenails). Thus their results were not consistent with our findings. In addition, the biostatistical related association, if applicable, does not appear to effect all metal biomarkers uniformly as thallium and cadmium were not associated with measured creatinine clearance in the current analysis nor was antimony in a previous analysis of lead workers herein (Chapter 3).

In conclusion, we found the significant associations between *ln*-urine uranium and measured creatinine clearance and NAG present only in models that used urine creatinine to adjust for dilution. In our analysis with total uranium excreted, an approach to address urine dilution that is less commonly used in occupational and environmental studies, these associations

were not observed. The implications of these findings are widespread if the observed associations are biostatistical rather than biological as it could affect the use and interpretation of all biomarkers that are adjusted for creatinine in kidney research. Future nephrotoxicity studies involving creatinine adjusted biomarkers should also consider non-creatinine based methods of adjustment as well as additional non-urine based exposure measures (e.g. blood levels).

Table 4-1. Selected demographic, exposure, and health outcome measures in 684 lead workers

Characteristic	N (%)	
Male	535 (78.2)	
Current workers	450 (65.8)	
Diabetes (yes)	25 (3.7)	
Hypertension (yes)	84 (12.3)	
Current smokers	294 (43.0)	
	Median	**Mean (SD)**
Age, years	46.5	47.6 (8.0)
BMI, kg/m2	24.1	24.2 (2.9)
Systolic blood pressure, mm Hg	121.5	123.6 (15.7)
Diastolic blood pressure, mm Hg	74.5	75.1 (12.1)
Blood lead, µg/dl	21.5	23.2 (14.3)
Tibia lead*, µg Pb/g bone mineral	20.0	27.1 (29.3)
Uranium, µg/g creatinine	0.07	0.13 (0.18)
Uranium excreted, µg	0.02	0.03 (0.04)
Thallium, µg/g creatinine	0.39	0.44 (0.23)
Thallium excreted, µg	0.09	0.10 (0.06)
Cadmium,µg/g creatinine	0.83	1.0 (0.6)
Cadmium excreted, µg	0.19	0.21(0.10)
MDRD GFR, ml/min/1.73 m2	95.8	97.7 (19.4)
Cystatin-C-based eGFRm, ml/min/1.73 m2	112.7	112.0 (17.8)
Measured creat. clearance, ml/min	110.4	110.8 (30.9)
NAG µmol/h/g	318.1	386.3 (282.1)

*n=678

Table 4-2. Spearman correlation coefficients among urine metals and blood and tibia lead in 684 lead workers

	Uranium, µg/g creat.	Uranium exc, µg	Thallium, µg/g creat.	Thallium exc, µg	Cadmium, µg/g creat.	Cadmium exc, µg	Blood lead, µg/dL
Uranium exc, µg	0.95#						
Thallium, µg/g creat.	0.09*	0.06					
Thallium exc, µg	- 0.01	0.12**	0.79#				
Cadmium, µg/g creat.	0.04	- 0.09*	0.21#	-0.10*			
Cadmium exc, µg	- 0.04	- 0.01	0.17#	0.20#	0.80#		
Blood lead, µg/dL	- 0.21#	- 0.15#	0.17#	0.27#	-0.12*	-0.01	
Tibia lead, µg/g bone	- 0.03	- 0.04	0.16#	0.10*	0.08#	0.05	0.47#

*p-value < 0.05; **p-value < 0.01; #p-value < 0.001

Table 4-3. Associations of urine uranium and kidney outcomes in 684 lead workers

Kidney Outcome	Model 1 *Ln*-Uranium μg/L[a] β coeff (95 % CI)	Model 2 *Ln*-Uranium μg/g creatinine β coeff (95 % CI)	Model 3 *Ln*-Uranium excreted, μg β coeff (95 % CI)
MDRD eGFR, ml/min/1.73 m^2	0.02 (-1.0, 1.1)	0.03 (-1.0, 1.1)	-0.2 (-1.2, 0.9)
Cystatin-C-based eGFRm, ml/min/1.73 m^2	-0.5 (-1.3, 0.4)	-0.5 (-1.3, 0.4)	-0.3 (-1.1, 0.6)
Measured Creatinine Clearance, ml/min	-2.3 (-3.9, -0.6)**	-2.7 (-4.4, -1.1)**	0.2 (-1.2, 1.7)
***Ln*-NAG, μmol/h/g creatinine**	0.037 (0.004, 0.071)*	0.039 (0.006, 0.073)*	0.025 (-0.010, 0.060)

*p-value < 0.05; **p-value<0.01

[a]Models adjusted for age, gender, BMI, employment status (current vs. former lead worker), enrollee status (phase I vs. II study entry), annual income (10, 10-20, 20-30, 30-40, and > 40 million won), education (< middle school graduate, < high school graduate, high school graduate, > high school), alcohol consumption (never, former, current), smoking dose [(cigarettes per day × years of smoking) in quartiles for current smokers and ex-smoker status], diastolic blood pressure, blood lead, tibia lead and, *ln*-urine thallium, *ln*-cadmium and *ln*-urine creatinine (model 1 only)

Table 4-4. Occupational and epidemiological studies examining glomerular filtration measures as outcomes

Author (Year), Location Study Population	Study Design	Exposure Assessment	Filtration Measures & Findings
Thun, et al. (1985), Colorado[115] Current and former uranium mill workers (n=39) with at least 1 year of work in the yellowcake area and cement plant worker controls (n=36)	Chi-square test was used to examine differences between exposed and control participants for dichotomous variables. Matched pair Student t-test (matched on race, sex age [± 2 yrs]) was used for continuous variables	Most mill workers (n=27) were exposed to "yellowcake", a powder containing salts and oxides of NU ammonium diuranate (soluble); a small group of others mill workers (n=12) worked in the crushing area where they were exposed to less soluble uranium Mean blood lead (μmol/L) in exposed was 0.45 vs. 0.78 in controls ($p < 0.001$; mean blood cadmium (nmol/L) in exposed was 31.2 vs. 24.7 in controls ($p > 0.05$)	8-h urine collection for urine creatinine Serum β2 microglobulin was higher in the exposed group; In contrast serum creatinine was lower ($p=0.02$) and measured creatinine clearance was higher ($p=0.04$; matched analysis) in the exposed group ; these differences are in the opposite direction from that expected in a traditional nephrotoxic pattern. The authors note that in the reference group, creatinine clearance was correlated with blood lead ($r_s=0.32$, $p=0.057$)
Kurrtio, et al. (2002), Southern Finland[109] General population (ages 15-82) currently drinking well water in an area of known high uranium water concentrations (n=325) Persons with diabetes excluded (n=4)	Uranium in μg/g creatinine used in generalized linear regression models that adjusted for age, sex and BMI Adjustment for duration of uranium exposure, education, occupation, analgesic use, or smoking (never, ex-smoker, or current smoker) in linear models did not affect uranium exposure associations with kidney functions	Oral exposure to NU in drinking water; wells used as the main source of drinking water for an average of 13years (range 1–34 years) Uranium in drinking water: median 28 μg/L (interquartile range (IQR) 6–135, maximum 1,920 μg/L) Residents collected overnight urine samples for uranium measurement in plastic bottles and recorded collection times (median 11 hr, range 7–17 hr). Uranium in urine: median 13 ng/mmol creatinine (IQR 2–75 ng/mmol creatinine)	No association with measured creatinine clearance from the same overnight urine samples used for uranium assessment Creatinine clearance calculated as: ([Urine creatinine · Urine volume]/Serum creatinine)/Urine collection time(1.73/body surface)

	and were not used as covariates.	Median daily uranium intake of 39 μg (IQR 7–224 μg)	
Pinney, et al. (2003), Ohio[114] Residents who lived within 5 miles of a uranium processing plant for at least 2 years from 1/1/52- 12/18/84 (n=8464)	Linear regression adjusted for age and sex	Oral exposure to NU via drinking water and unknown uranium type(s) exposure from plant resulting in inhalation via airborne emissions and oral exposures via contaminated food and drinking water Exposure variables: 1) residence proximity to plant (≤ 2 miles vs. > 2 miles); 2) water source (well/cistern or municipal)	Increased serum creatinine in category of residents living within 2 miles of the plant compared to category of residents living greater than 2 miles from plant
Kurrtio et al, (2006), Southern Finland[110] Subset of Kurrtio, et al. (2002)[109] ages 18 to 81 who still used the same well for drinking water (n=193)	Log-transformed urine uranium (μg/L) used in linear regression models that adjusted for sex, age, BMI, smoking, and analgesic use	Oral exposure to NU in drinking water; wells used as the main source of drinking water for an average of 16 years (range 5-40 years) Uranium concentration in drinking water: median 25 μg/L (IQR 5 to 148 μg/L; maximum, 1,500 μg/L) Urine collection for uranium as in Kurrtio, et al, (2002); median collection time 8 hours (range 5-12 hours) Uranium concentrations in urine were reported to be 44% greater on average than in Kurrtio (2002); actual values were not provided Uranium dose biomarkers also included hair and toenail uranium concentrations	No association of urine uranium (μg/L) with NAG, serum cystatin-C, or measured creatinine clearance obtained as in Kurrtio (2002) Authors note results were similar with creatinine correction of urinary uranium as well as for other uranium exposure indicators: uranium in drinking water, hair, toenails, and daily and cumulative uranium intake (data were not shown)
McDiarmid et al (2000, 2001, 2004, 2006, 2007, 2009)[111-113, 116-118] Cohort of Gulf War Veterans that were exposed to DU	Differences in outcome measures between high low urine uranium exposure groups were examined using the Mann–Whitney *U*-test	DU Exposures include: acute inhalation exposure to DU particles; acute dermal exposure to DU particles in wounds, and chronic endogenous exposure to embedded DU shrapnel	24-hour urine collection for and creatinine 2006, 2007, 2009 – No differences in NAG 2000, 2001, 2007 - No differences in

munitions by "friendly-fire" in 1991 ' Participants were divided in to high and low uranium exposure groups based on urine uranium levels (high group is >0.10 µg/g creatinine) 2000- high (n=14) low (n=53) 2001- high (*n=13)* low (*n*= 37) 2004- high (n =13) low (n=26) 2006- high (n=13) low (n=18) 2007- high (n=10) low (n=24) 2009 high (*n* = 10) low (*n* = 25)		24-hour urine collection for total uranium (µg/g creatinine) 2009 - Blood uranium also collected; total urine uranium and blood uranium closely correlated only when urine uranium concentrations were above 0.1 µg/g creatinine	serum creatinine 2004, 2006 - Serum creatinine higher in low exposure group. (p-value(s) = 0.03 in 2004 and 0.11 in 2006) 2009 - Serum creatinine higher and GFR lower in the low exposure group (p=0.06, p=0.11, respectively), and although not statistically significant, creatinine clearance in the low U group was approximately 20% lower than the high U group Differences between exposure groups observed in 2004, 2006, and 2009 are opposite of what would be expected in a traditional nephrotoxic pattern

Chapter 5: DISCUSSION

Summary of Findings

The first objective of this study was to evaluate associations between occupational exposures to antimony and thallium and kidney outcomes, and to evaluate the impact of environmental cadmium exposure on those associations in a population of workers occupationally exposed to lead. This was followed by an evaluation of the associations between environmental uranium exposure and kidney outcomes in the same population of lead workers. We addressed these objectives in two cross-sectional analyses of data from an existing cohort of 684 currrent and former lead workers from the Republic of Korea. The results for each of the analyses are summarized in Table 5-1 and are discussed below according to the specific aims of this study.

The specific aims of this cross-sectional study were:

1. To examine associations between clinical kidney outcome measures (serum creatinine, serum cystatin c, calculated creatinine clearance, measured creatinine clearance, and serum creatinine- and cystatin-C-based estimated glomerular filtration rates (eGFR)) and urine concentrations of antimony, thallium, and uranium;
2. To examine associations between a potential biomarker of early nephrotoxic effects (N-acetyl-β-glucosaminidase), and urine concentrations of the above metals; and
3. To evaluate the impact of urine cadmium on these associations.

To achieve these aims, we focused our analyses separately according to primary source of exposure – occupational or environmental.

In Manuscript I, as reported in Chapter 3, we examined associations of *ln* -transformed urine antimony and thallium concentrations with a range of traditional kidney outcomes, including eight serum creatinine- and cystatin-C-based glomerular filtration measures and NAG, a proximal tubular early biological effect marker. After adjustment, higher urine concentrations of antimony and thallium, in separate models, were significantly associated with higher NAG, a

direction consistent with nephrotoxicity. In contrast, *ln*-urine antimony was associated with serum creatinine-based glomerular filtration measures in the direction opposite that expected in traditional nephrotoxicity and *ln*-urine thallium was associated with both serum creatinine and cystatin-C-based glomerular filtration measures also in a direction opposite that expected in traditional nephrotoxicity. Neither metal was associated with measured creatinine clearance.

Associations with each study metal were also evaluated following additional adjustment for the other metal and ln-urine cadmium (Table 3-3). Both antimony and thallium associations with NAG were attenuated by cadmium adjustment. Antimony associations with traditional kidney outcomes also became non-significant after thallium and cadmium adjustment. However, thallium associations remained significant, and with the exception of the combined creatinine- and cystatin-C-based eGFR, beta coefficients for thallium associations with cystatin-C-based filtration measures became stronger with additional adjustment.

In Manuscript II, as discussed in Chapter 4, we examined associations of *ln* -transformed urine uranium concentrations with a three clinical kidney outcomes (measured creatinine clearance and serum creatinine- and cystatin-C-based glomerular filtration measures) and NAG. In linear regression models, urine uranium dose was evaluated by three different methods to adjust for urine dilution: the traditional approach, in which the metal concentration is divided by urine creatinine; a more recent approach in which the urine metal and creatinine are included as separate covariates in the model[79] and; a non-creatinine based approach where urine dilution adjustment was achieved by entering the metal as total amount excreted (μg) over the four-hour urine collection period. This latter approach is an option only because the metals were measured from a timed urine collection, an approach that is rarely available in the context of a large epidemiological study. After adjustment for kidney risk factors (as in Manuscript I), lead dose, and *ln*-urine thallium and cadmium, higher *ln*-urine uranium was associated with lower measured creatinine clearance and higher NAG, a pattern consistent with traditional nephrotoxicity.

However, these associations were present only in the two models that used urine creatinine to adjust for urine dilution (Table 4-3). Uranium was not associated with either creatinine- or cystatin-C-based eGFR in any of the three models. These results were consistent in fully adjusted models that did not include urine thallium or cadmium.

Unexpected Findings

The results of this study indicate that interpretation of low-level urine metals data may be more complex than previously appreciated. We observed statistically significant associations in patterns that are unique to each metal (Table 5-1). Urine thallium was associated with higher levels of both serum creatinine- and cystatin-C-based eGFRs, a pattern opposite that expected in traditional nephrotoxicity. Similarly, urine antimony and cadmium were also positively correlated with serum creatinine-, but not serum cystatin-C-based eGFRs. In contrast, urine uranium was associated with only one glomerular filtration measure, measured creatinine clearance, and in the direction consistent with nephrotoxicity. However, this association was only present if urine dilution was adjusted though use of urine creatinine.

Table 5-1. Summary of significant urine metal associations with select kidney outcomes in Korean lead worker cohort

Urine Metal	Serum creatinine-based eGFR	Serum cystatin-C-based eGFR	4-hour measured creatinine clearance	NAG
Antimony	Positive[a]	–	–	
Thallium	Positive	Positive	–	
Uranium	–	–		
Cadmium	Positive	–	–	

"Positive" or "Negative" - direction of association
direction of association opposite that expected in traditional nephrotoxicity
direction of association expected in traditional nephrotoxicity
[a] association attenuated by cadmium adjustment
* association observed only if adjusted for urine dilution using urine creatinine

Several potential hypotheses were considered to explain the novel findings of our study. The paradoxical direction of thallium associations with both serum creatinine-and cystatin-C-based glomerular filtration measures is consistent with a kidney function effect such as reverse causality or thallium-related hyperfiltration. Reverse causality, which implies that lower urine thallium levels reflect less metal excretion because of decreased kidney function, is a plausible explanation because higher *ln*-urine thallium was associated only in the group of lead workers with lower kidney function in models stratified at the median outcome measure (e.g. eGFR). For this reason, thallium-related hyperfiltration, a longitudinal process where an initial rise in glomerular filtration rate is followed by a decline indicative of chronic kidney disease, seems less likely. However, the two populations stratified by median outcome have a number of differences in terms of age, sex and lead work status. Thus we cannot entirely exclude hyperfiltration based on the results of these stratified models.

In contrast, a biomarker specific hypothesis is more relevant for antimony and cadmium where unexpected associations were observed only with serum creatinine- and not cystatin-C-based filtration measures. In this regard, a statistical association related to the use of urine creatinine in both exposure and outcome metrics is a consideration, where the positive correlation between urine creatinine used to adjust urine metal levels for urine dilution, and serum creatinine, used as an outcome and to estimate other kidney outcomes, spuriously altered the observed associationsThe remarkable change in urine creatinine associations that were observed when metals strongly associated with kidney outcomes were added to the model lends support for this hypothesis. The fact that urine uranium levels were significantly associated with measured creatinine clearance and NAG only when urine creatinine was included in the regression model also suggests that these results may be, in part, due to a biostatistical association. The same urine creatinine measurement used to adjust urine uranium levels for urine dilution was also used to calculate measured creatinine clearance; this four-hour urine creatinine measurement was also correlated with the spot urine creatinine used to adjust NAG for urine dilution.

Given the different patterns of associations between metals, hypotheses related to unique metal-specific mechanisms, such as alterations in metal excretion due to protein binding,[7] remain considerations. Metals are bound to various proteins during transport in the serum and renal transporters are involved in excretion of these proteins[119] which may influence urine metal levels. Cadmium binding to metallothionen is the most widely recognized, however it is possible for other metals to bind to this protein.[119] Specific lead-binding proteins, anagolous to metallothionein, have also been identified.[120] Mercury, cobalt and several other metals bind to albumin,[119][121] and nephrotoxic effects, such as increased serum creatinine, have been observed in animals exposed to high doses of cobalt,[122, 123] an essential metal contained in vitamin B12.

However, we were able to exclude other potential hypotheses. Associations in directions opposite that expected are not likely due to lead-related hyperfiltration because lead was included as a covariate in the regression models and moreover, thallium was also positively associated with MDRD eGFR in former lead workers whose blood lead levels were negatively associated with MDRD eGFR, a pattern consistent with traditional lead-related nephrotoxicity. We were also able to exclude metal collinearity through examination of variance inflation factors in fully adjusted models; and in addition, the paradoxical direction of associations were also evident in the more simple *a priori* models.

Novel results were also observed in the multiple metals analysis. Although common in environmental exposure, analysis of mixtures is infrequently evaluated in research. Co-exposures to metals with common target organs may have additive or multiplicative effects. A recent analysis of 1999-2006 NHANES data found that increased blood cadmium and lead levels were independently associated with increased prevalences of albuminuria and reduced eGFR [100]. Furthermore, odds of having both outcomes were four-fold higher in participants in the highest quartiles of both metals compared to those in the lowest. However, in the current analysis, attenuation was observed. In particular, NAG is routinely used in nephrotoxicant research. If we

had limited our analysis to associations between antimony and thallium in separate models of NAG, we would have concluded that the results supported nephrotoxicity from both metals at low environmental levels of exposure. Instead, our multiple metal analysis allowed us to identify cadmium as the primary toxicant for NAG. The antimony attenuation observed in our analysis may be related to measurement uncertainty, given the large proportion of samples below the MDL, or to population differences due to higher exposure in the lead battery workers.

Limitations

Limitations are related to the study design, measurement constraints, and the population. CKD is a progressive disease, and the cross-sectional design limits our ability to make causal inferences as it is not possible to establish a temporal relation. However, this project is part of a larger longitudinal cohort which affords the opportunity to address this limitation in subsequent analyses.

Unknown or unmeasured variables can also affect our inferences. For example, it was not anticipated that metals other than cadmium would be analyzed at the time of urine collection in the larger cohort study; therefore, samples were not collected with the preservative required to evaluate urine mercury levels. Measurements of other potentially nephrotoxic metals (e.g. beryllium, arsenic) were not obtained due to limitations associated with the assays. However, advances in analytic techniques may allow for future analysis.

Another limitation is that our population is entirely Korean as well as both occupationally and environmentally exposed to a mixture of metals. However, an association between higher urine cadmium and higher eGFR has also been observed in the NHANES population (Tellez-Plaza, et al. manuscript in preparation), which indicates that results of this study are relevant to general populations. We were also not able to assess the effects of race or ethnicity. Yet the population is diverse in other ways (e.g. gender, age, renal risk factors) that are relevant to any population at risk for CKD.

Strengths

This study has several important strengths. The parent longitudinal cohort study of lead and cadmium nephrotoxicity was conducted by a multi-disciplinary research team with substantial expertise and is the largest and longest study of renal function in lead workers to date. The urinary metals panel was performed by an experienced certified laboratory using the currently preferred technique. The panel approach is much more cost effective than a single metal by metal approach. The cost savings allowed us to measure multiple metals in the entire eligible population. As a result, the large sample size (n=684) afforded us power to detect relatively small associations.

Actual measurement of GFR is not feasible in large epidemiological studies such as this. The preferred approach in such research is to use serum levels of endogenous compounds in equations to estimate GFR. Serum creatinine is the most widely used compound for this purpose, however its level may be impacted by non-kidney factors, such as muscle mass. While other studies may rely solely on this commonly used measure, we considered a range of outcomes that afforded us the opportunity to assess consistency in results across different measures of glomerular filtration. Among these was a GFR estimating equation based on an alternative biomarker to serum creatinine, serum cystatin C, which has been an emerging focus in the kidney research community because it may be less impacted by non-kidney factors.[62]

Conclusions and Implications

Nephrotoxicity associated with mixed metal exposures, and its contribution to the overall incidence of CKD, has been inadequately studied, despite the fact that humans are commonly exposed to mixtures of metals. Metals, such as lead and cadmium, are established nephrotoxicants. However, relatively little is known about the nephrotoxic effects of occupational or environmental exposures to other ubiquitous metals such as antimony, thallium, and uranium. To our knowledge, this study is the first to consider the nephrotoxic effects of

antimony, thallium, uranium, and cadmium co-exposures in a population occupationally exposed to lead. Novel results were observed in the multiple metals analysis which revealed attenuation of observed associations by other metals, in particular cadmium. NAG is routinely used in nephrotoxicant research. If our analysis was limited to associations between antimony and thallium in separate models of NAG, we would have concluded that both metals are nephrotoxic at low environmental levels of exposure. Instead, our multiple metal analysis allowed us to identify cadmium as the primary toxicant for NAG.

Urine creatinine is widely used to adjust for urine dilution in kidney biomarker research. To our knowledge, this is the only study that compares associations between urine uranium and kidney outcomes using three different approaches to adjust for urine dilution, including the unique metric based on a timed urine collection. If we had not also considered total uranium excreted (μg) over the 4-hour collection period as an alternate method of adjustment for urine dilution, we would have concluded that environmental uranium exposure is associated with lower measured creatinine clearance and higher NAG and thus indicative of nephrotoxicity.

Suggestions for Future Research

Our novel findings have important implications for future studies. Additional research is needed to determine the mechanism(s) for associations between higher urine metal concentrations and higher glomerular filtration measures and differences in associations by method of adjustment for urine dilution. In such work, kidney research that uses both creatinine- and non-creatinine-based outcomes should explore non-creatinine based methods of adjustment for urine dilution as well as non-urine based exposure measures (e.g., blood levels).

The importance of a multiple metals analysis was revealed in the attenuation of observed associations by other metals. Therefore, co-exposures to other nephrotoxic agents should be considered whenever possible as single metal analyses may increase the potential for inaccurate risk conclusions.

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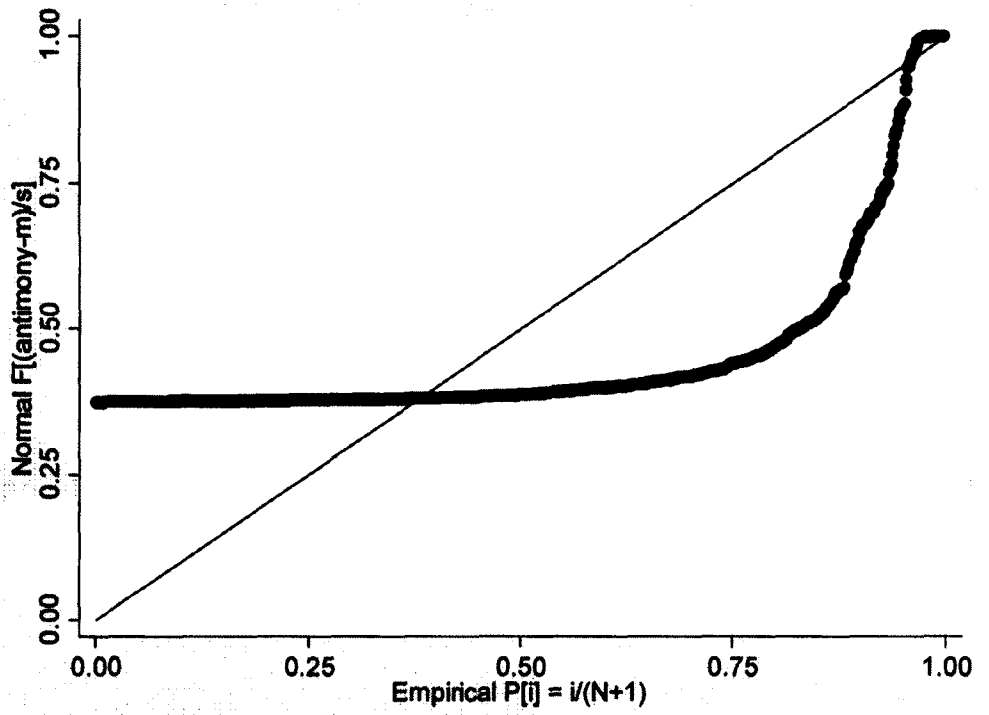
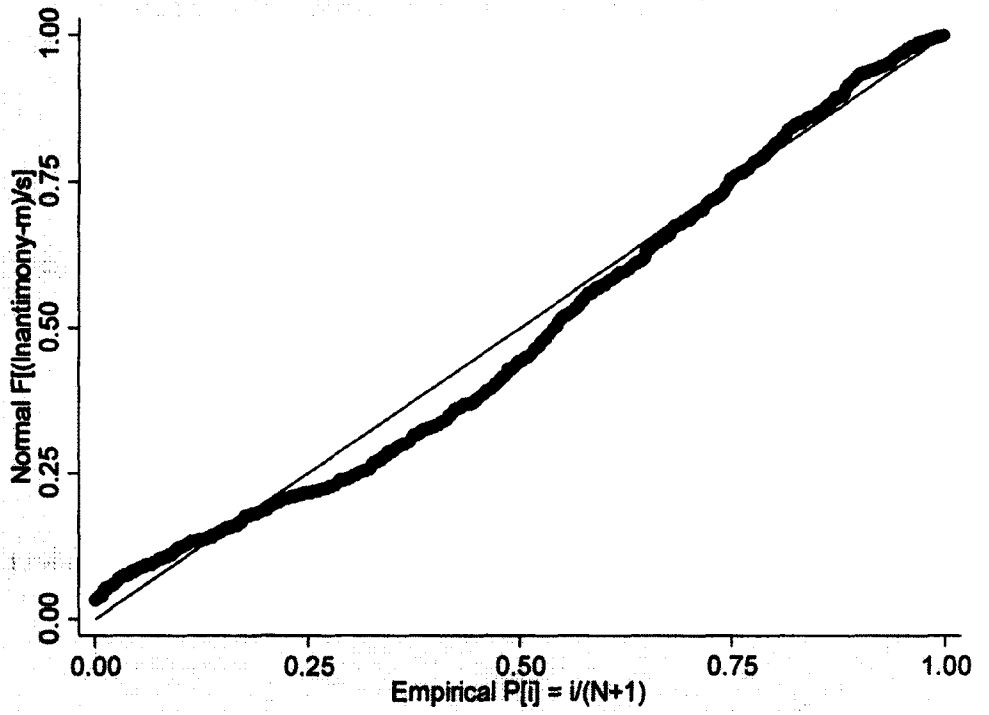
APPENDICES, CHAPTER 3

Appendix Figures 3-1 a-j. Antimony, thallium, and NAG normal probability plots pre and post log transformation

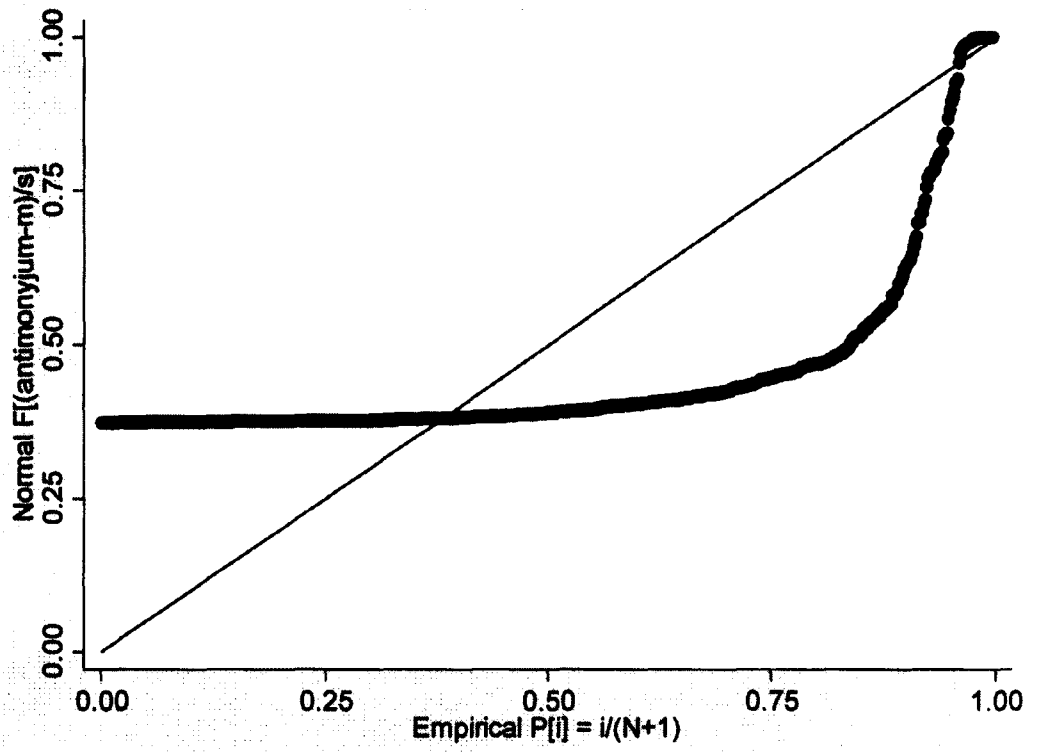
a. Urine Antimony (µg/L) Normal Probability Plot



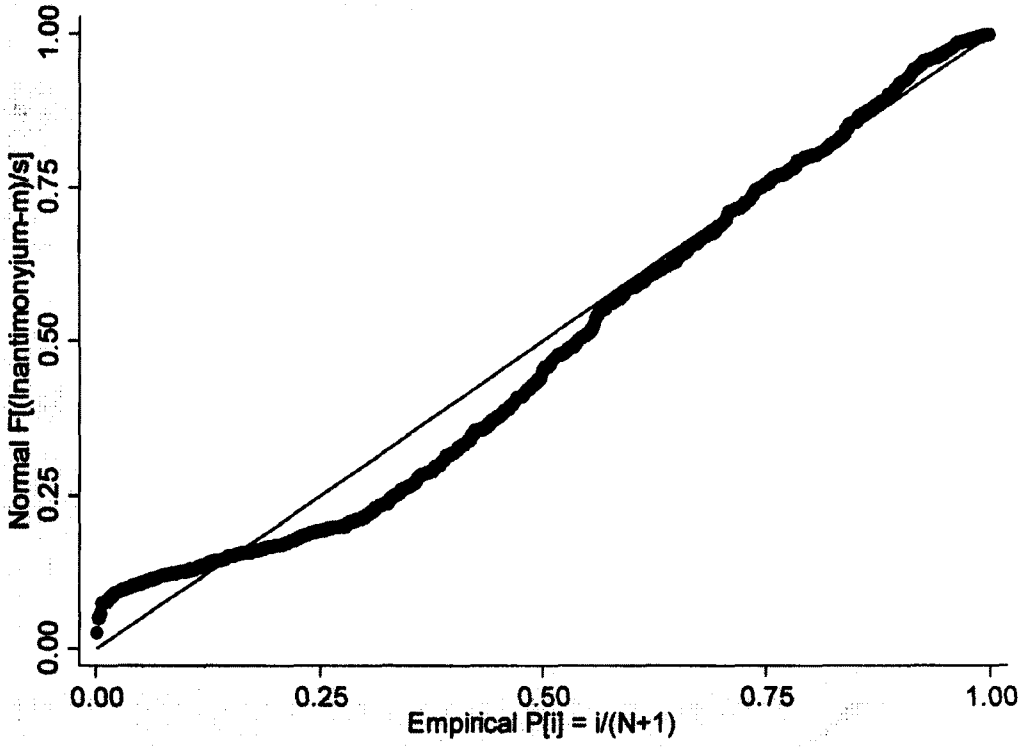
b. Ln-urine Antimony (µg/L) Normal Probability Plot



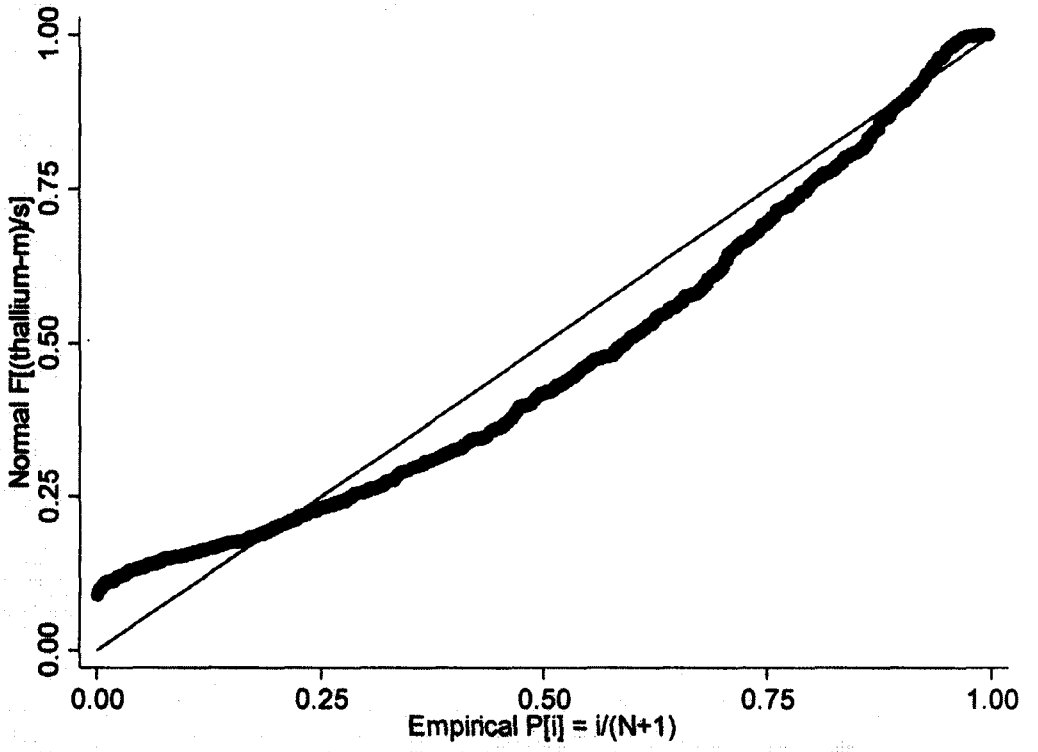
c. Urine Antimony (µg/g creatinine) Normal Probability Plot



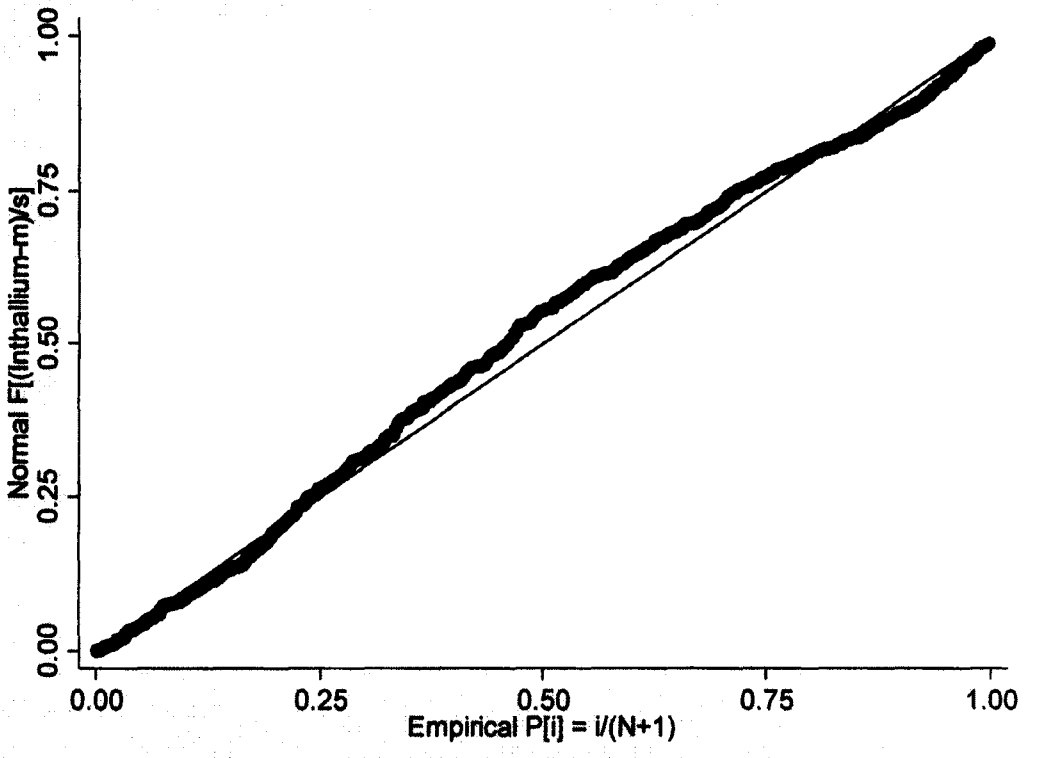
d. Ln-urine Antimony (µg/g creatinine) Normal Probability Plot



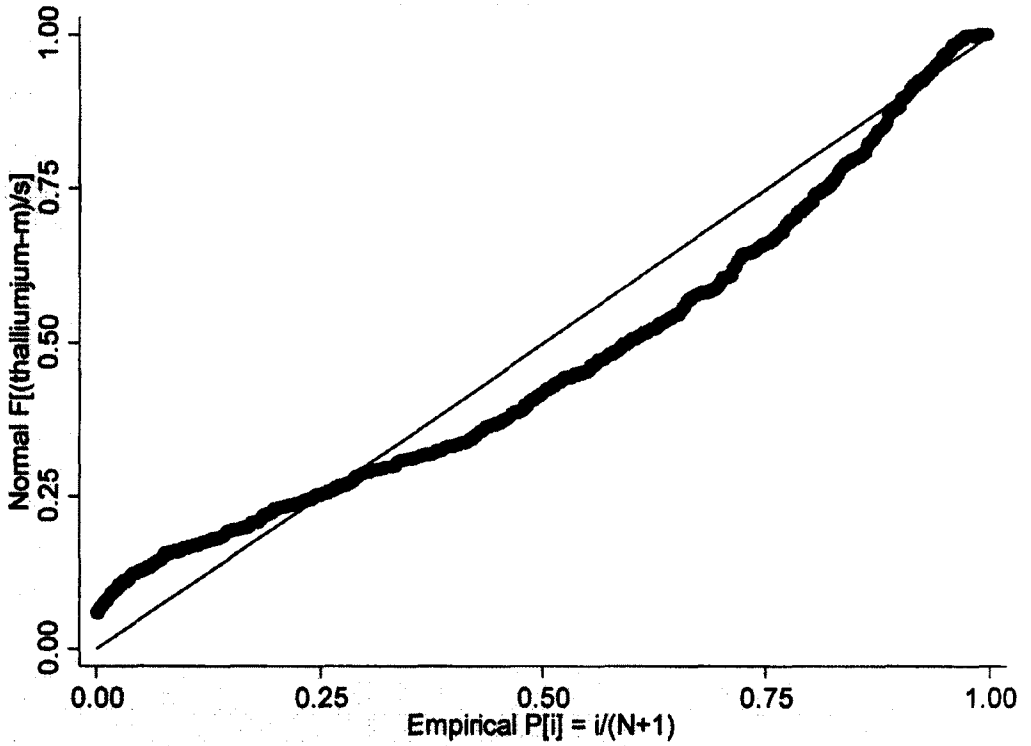
e. Urine Thallium (µg/L) Normal Probability Plot



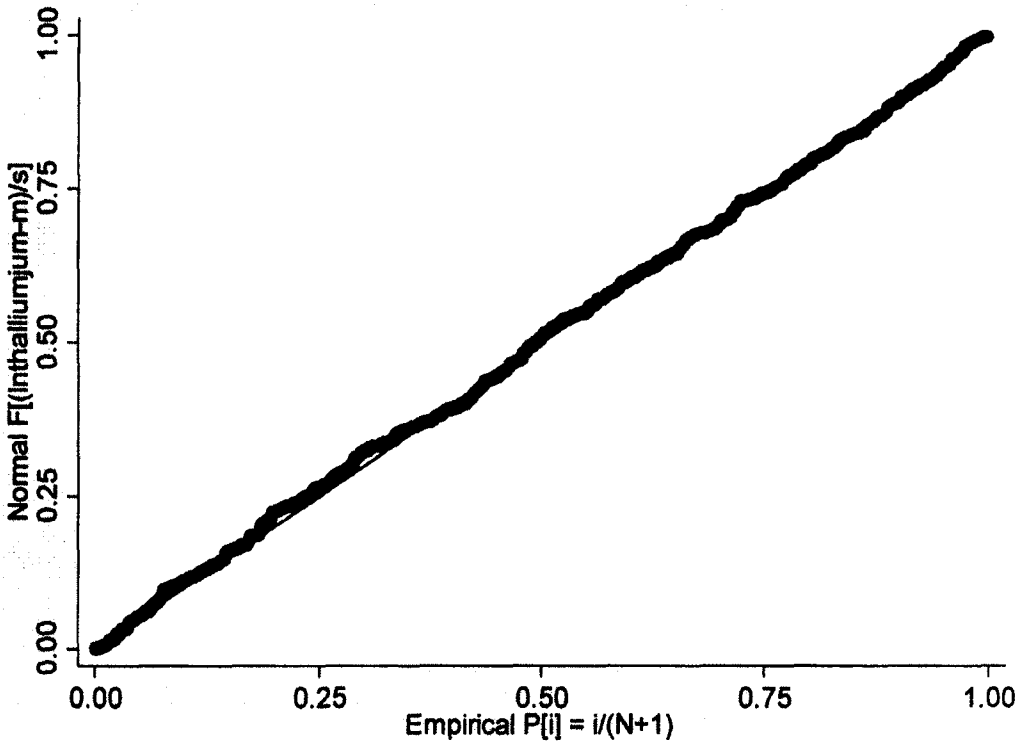
f. Ln-urine Thallium (µg/L) Normal Probability Plot



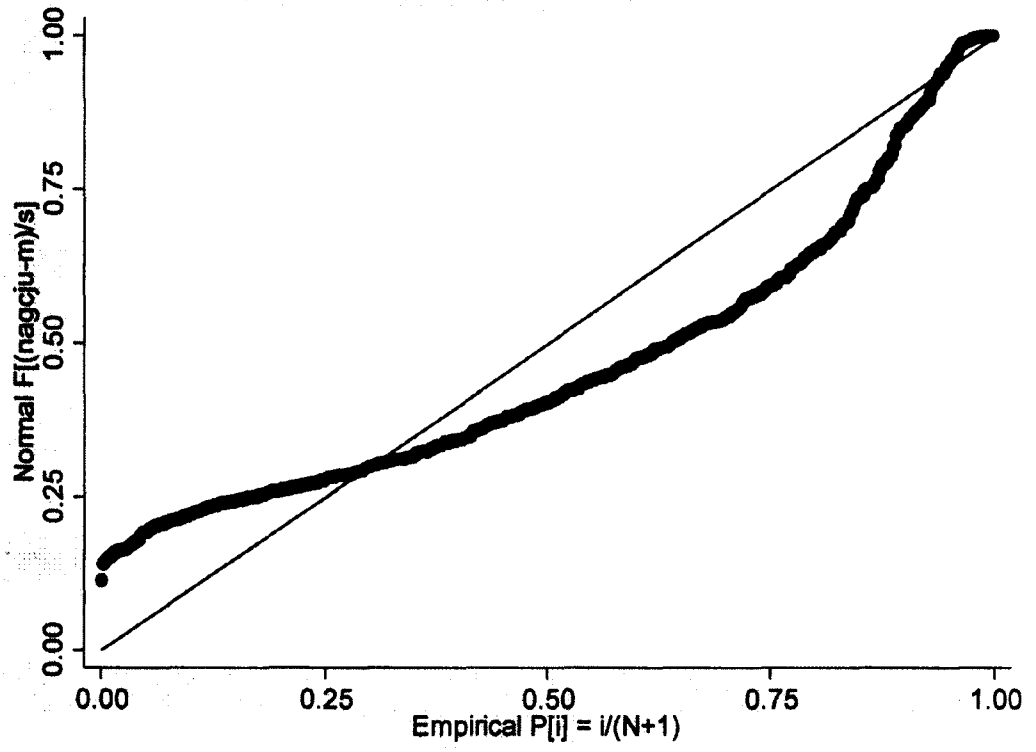
g. Urine Thallium (μg/g creatinine) Normal Probability Plot



h. Ln-urine Thallium (μg/g creatinine) Normal Probability Plot



i. NAG (μmol/h/g creatinine) Normal Probability Plot



j. Ln-NAG (μmol/h/g creatinine) Normal Probability Plot

Appendix Table 3-1. Urine antimony levels (ug/g creatinine) by plant type and worker status

Osan	89	1.79	64	4.67	25	0.32
Union (retired)	73	0.041	0	n/a/	73	0.08
Namil	52	0.387	30	1.04	22	0.12
Hankook	153	0.964	123	2.00	30	0.22
Sambu	15	0.162	0	n/a/	15	0.17
Samheung	9	0.279	3	0.90	6	0.21
Gunmyung	3	0.204	3	0.26	0	n/a
Donghae	23	2.21	19	3.12	4	2.45
Delko	47	0.038	47	0.32	0	n/a
Sebang	20	0.385	20	0.61	0	n/a
E- Power	2	0.213	2	0.25	0	n/a
Gunchun	23	0.145	23	0.15	0	n/a
Dongho	44	0.09	31	0.10	13	0.08
Sungnam	17	0.01	0	n/a/	17	0.10
Sangsin	4	1.024	3	2.14	1	0.07
Daehan	23	0.368	17	0.74	6	0.17
Samsung	2	0.439	0	n/a/	2	0.54
Sangsin Yeongong	9	0.77	9	0.75	0	n/a
Samgi	13	0.968	12	1.34	1	0.09
Heungkook	3	5.94	3	5.94	0	n/a
Yusin	1	0.10	0	n/a/	1	0.10
Dongyun	6	0.08	5	0.08	1	0.09
Dansuk	40	0.10	34	0.09	6	0.20
Doosan	4	0.07	0	n/a/	4	0.07
Hanil	1	0.13	1	0.13	0	n/a
Gawbong	7	0.50	0	n/a/	7	0.09
Kiyoung	1	0.04	1	0.04	0	n/a

Appendix Table 3.2. Urine Thallium levels (ug/g creatinine) by Plant Type and Name and Worker Status

Osan	89	0.406	64	0.402	25	0.415
Union (retired)	73	0.274	0	n/a	73	0.274
Namil	52	0.404	30	0.379	22	0.440
Hankook	153	0.531	123	0.533	30	0.524
Sambu	15	0.404	0	n/a	15	0.404
Samheung	9	0.520	3	0.596	6	0.486
Gunmyung	3	0.714	3	0.714	0	n/a
Donghae	23	0.359	19	0.340	4	0.466
Delko	47	0.277	47	0.277	0	n/a
Sebang	20	0.319	20	0.319	0	n/a
E- Power	2	0.133	2	0.133	0	n/a
Gunchun	23	0.285	23	0.285	0	n/a
Dongho	44	0.302	31	0.307	13	0.289
Sungnam	17	0.372	0	n/a	17	0.372
Sangsin	4	0.495	3	0.398	1	0.955
Daehan	23	0.420	17	0.450	6	0.344
Samsung	2	0.274	0	n/a	2	0.274
Sangsin Yeongong	9	0.287	9	0.287	0	n/a
Samgi	13	0.451	12	0.431	1	0.778
Heungkook	3	0.387	3	0.387	0	n/a
Yusin	1	0.609	0	n/a	1	0.609
Dongyun	6	0.341	5	0.366	1	0.240
Dansuk	40	0.444	34	0.457	6	0.376
Doosan	4	0.311	0	n/a	4	0.311
Hanil	1	0.333	1	0.333	0	n/a
Gawbong	7	0.357	0	n/a	7	0.357
Kiyoung	1	0.660	1	0.660	0	n/a

Appendix Table 3-3. Associations between urine thallium and antimony and kidney outcomes in single metal a priori models

Kidney Outcome	Ln-urine Thallium (µg/L) a priori model β coeff (95 % CI))	Ln-urine Antimony (µg/L) a priori model β coeff (95 % CI))
Serum Creatinine, mg/dl	-0.059 (-0.079, -0.039)#	-0.012 (-0.018, -0.006)#
Calculated Creatinine Clearance, ml/min	5.2 (2.8, 7.7)#	1.0 (0.2, 1.8)*
MDRD eGFR, ml/min/1.73 m^2	8.0 (5.2, 10.8)#	1.5 (0.6, 2.3)**
Measured Creatinine Clearance, ml/min	4.4 (0.3, 8.5)*	0.2 (-1.0, 1.5)
Serum Cystatin C, mg/L	-0.037 (-0.049, -0.025)#	-0.005 (-0.009, -0.001)**
CYSeGFRs, ml/min/1.73 m^2	7.0 (4.6, 9.4)#	0.8 (0.01, 1.5)*
CYSeGFRm, ml/min/1.73 m^2	6.5 (4.2, 8.8)#	0.6 (-0.1, 1.3)[a]
Combined CYSeGFRc, ml/min/1.73 m^2	7.9 (5.5, 10.3)#	1.2 (0.4, 1.9)**
***Ln*-NAG, µmol/h/g creatinine**	0.074 (-0.006, 0.154)[a]	0.018 (-0.006, 0.043)

Models adjusted for age, gender, BMI, ln-urine creatinine

[a]p-value <0.01; *p-value < 0.05; **p-value < 0.01; #p-value < 0.001

Appendix Table 3-4. Associations between urine metals (μg/g creatinine) and kidney outcomes in 684 lead workers

Kidney Outcome	Model 1 β coeff (95 % CI))	Model 2 β coeff (95 % CI))	Model 3 β coeff (95 % CI))	Model 4 β coeff (95 % CI))
MDRD eGFR, ml/min/1.73 m^2				
Ln-Urine Antimony	1.5 (0.5, 2.5)**		0.9 (-0.03, 1.9)	0.8 (-0.2, 1.7)
Ln- Urine Thallium		6.3 (3.5, 9.0)#	5.8 (3.1, 8.6)#	4.6 (1.8, 7.4)**
Ln-Urine Cadmium				6.6 (3.5, 9.8)#
Calculated Creatinine Clearance, mL/min				
Ln-Urine Antimony	0.8 (-0.1, 1.6)		0.5 (-0.3, 1.4)	0.5 (-0.4, 1.3)
Ln- Urine Thallium		3.8 (1.3, 6.2)**	3.5 (1.1, 5.9)**	2.8 (0.4, 5.2)*
Ln-Urine Cadmium				3.3 (0.5, 6.1)*
Measured Creatinine Clearance, mL/min/1.73 m^2				
Ln-Urine Antimony	-0.7 (-2.2, 0.9)		-0.8 (-2.3, 0.7)	-0.6 (-2.1, 0.9)
Ln- Urine Thallium		0.8 (-3.4, 5.0)	1.2 (-3.1, 5.4)	1.9 (-2.4, 6.2)
Ln-Urine Cadmium				-4.7 (-9.6, 0.2)
Serum Creatinine, mg/dL				
Ln-Urine Antimony	-0.010 (-0.018,-0.003)**		-0.006 (-0.013, 0.001)	-0.005 (-0.01, 0.002)
Ln- Urine Thallium		-0.05 (-0.07, -0.03)#	-0.05 (-0.07, -0.03)#	-0.04 (-0.06, -0.02)#
Ln-Urine Cadmium				-0.05 (-0.07, -0.03)#
CYSeGFRm, mL/min/1.73 m^2				
Ln-Urine Antimony	-0.2 (-1.0, 0.7)		-0.5 (-1.3, 0.3)	-0.4 (-1.3, 0.4)
Ln-Urine Thallium		4.9 (2.7, 7.2)#	5.2 (2.9, 7.4)#	5.5 (3.2, 7.8)#
Ln- Urine Cadmium	-		-	-1.9 (-4.5, 0.8)

CYSeGFRs, mL/min/1.73 m^2				
Ln-Urine Antimony	-0.1 (-1.0, 0.8)		-0.5 (-1.4, 0.3)	-0.5 (-1.3, 0.4)
Ln-Urine Thallium		4.9 (2.6, 7.2)#	5.1 (2.8, 7.4)#	5.5 (3.1, 7.9)#
Ln- Urine Cadmium	-		-	-2.1 (-4.8, 0.6)
Combined eGFRc, mL/min/1.73 m^2				
Ln-Urine Antimony	0.9 (-0.01, 1.7)		0.4 (-0.5, 1.2)	0.3 (-0.5, 1.1)
Ln-Urine Thallium		6.3 (3.9, 8.6)#	6.1 (3.7, 8.5)#	5.5 (3.1, 7.9)#
Ln- Urine Cadmium	-		-	3.1 (0.4, 5.8)*
Serum Cystatin C, mg/L				
Ln-Urine Antimony	0.0003 (-0.004, 0.005)		0.002 (-0.002, 0.007)	0.002 (-0.002, 0.006)
Ln-Urine Thallium		-0.032 (-0.045, -0.020)#	-0.033 (-0.046, -0.021)#	-0.036 (-0.049, -0.024)#
Ln- Urine Cadmium				0.014 (-0.0002, 0.028)
***Ln*-NAG, µmoL/h/g creatinine**				
Ln-Urine Antimony	0.03 (0.004, 0.06)*		0.03 (-0.001, 0.06)	0.02 (-0.01, 0.05)
Ln-Urine Thallium		0.11 (0.03, 0.19)*	0.09 (0.01, 0.18)*	0.05 (-0.03, 0.13)
Ln- Urine Cadmium	-	-		0.23 (0.14, 0.32)#

Models also adjusted for age, gender, BMI, employment status (current vs. former lead worker), enrollee status (phase I vs. II study entry), annual income (10, 10-20, 20-30, 30-40, and > 40 million won), education (< middle school graduate, < high school graduate, high school graduate, > high school), alcohol consumption (never, former, current), smoking dose [(cigarettes per day × years of smoking) in quartiles for current smokers and ex-smoker status], diastolic blood pressure, blood lead, and tibia lead. Model 1 also adjusted for lead job duration

*p-value < 0.05; **p-value < 0.01; #p-value < 0.001

Appendix Table 3-5. Associations between urine antimony and thallium and kidney outcomes with and without urine cadmium adjustment in 684 lead workers

Kidney Outcome	Antimony Model 1 β coeff (95 % CI))	Antimony Model 2 β coeff (95 % CI))	Thallium Model 1 β coeff (95 % CI))	Thallium Model 2 β coeff (95 % CI))
MDRD eGFR, ml/min/1.73 m^2				
Ln-Urine Metal μg/L	1.5 (0.5, 2.5)**	1.2 (0.2, 2.2)*	6.8 (4.1, 9.6)#	5.5 (2.8, 8.3)#
Ln- Urine Cadmium μg/L	-	7.8 (4.6, 11.0)#	-	6.9 (3.8, 10.1)#
Calculated Creatinine Clearance, ml/min				
Ln-Urine Metal μg/L	0.8 (-0.1, 1.6)	0.6 (-0.2, 1.5)	4.0 (1.5, 6.5)**	3.2 (0.8, 5.6)**
Ln- Urine Cadmium μg/L	-	4.2 (1.4, 6.9)**	-	3.6 (0.9, 6.4)*
Measured Creatinine Clearance, ml/min				
Ln-Urine Metal μg/L	-0.6 (-2.1, 0.9)	-0.4 (-1.9, 1.0)	2.7 (-1.4, 6.8)	3.3 (-0.9, 7.4)
Ln- Urine Cadmium μg/L	-	-2.6 (-7.3, 2.1)	-	-3.5 (-8.3, 1.2)
Serum Creatinine, mg/dl				
Ln-Urine Metal μg/L	-0.01 (-0.02,-0.003)**	-0.01 (-0.02, 0.0004)*	-0.05 (-0.07, -0.03)#	-0.04 (-0.06, -0.02)#
Ln- Urine Cadmium μg/L	-	-0.06 (-0.08, -0.03)#	-	-0.05 (-0.07, -0.03)#
CYSeGFRm, ml/min/1.73 m^2				
Ln-Urine Metal μg/L	-0.2 (-1.0, 0.7)	-0.1 (-1.0, 0.7)	4.9 (2.6, 7.2)#	5.3 (2.9, 7.6)#
Ln- Urine Cadmium μg/L	-	-0.8 (-3.4, 1.9)	-	-2.1 (-4.7, 0.6)

CYSeGFRs, ml/min/1.73 m^2				
Ln-Urine Metal μg/L	-0.1 (-1.0, 0.7)	-0.1 (-1.0, 0.8)	5.3 (2.9, 7.7)#	5.7 (3.3, 8.1)#
Ln- Urine Cadmium μg/L	-	-0.7 (-3.5, 2.0)	-	-2.1 (-4.8, 0.6)
Combined eGFRc, ml/min/1.73 m^2				
Ln-Urine Metal μg/L	0.9 (-0.01, 1.7)	0.7 (-0.2, 1.5)	6.3 (3.9, 8.6)#	5.7 (3.3, 8.1)#
Ln- Urine Cadmium μg/L	-	4.3 (1.5, 7.0)**	-	3.2 (0.5, 5.9)*
Serum Cystatin C, mg/L				
Ln-Urine Metal μg/L	0.0004 (-0.004, 0.005)	0.0001 (-.005, 0.005)	-0.032 (-0.045, -0.02)#	-0.035 (-0.048, -0.022)#
Ln- Urine Cadmium μg/L	-	0.008 (-0.007, 0.022)	-	0.016 (0.001, 0.03)*
***Ln*-NAG, μmol/h/g creatinine**				
Ln-Urine Metal μg/L	0.03 (0.004, 0.06)*	0.02 (-0.01, 0.05)	0.10 (0.01, 0.18)*	0.05 (-0.03, 0.14)
Ln- Urine Cadmium μg/L	-	0.24 (0.14, 0.33)#	-	0.23 (0.14, 0.33)#

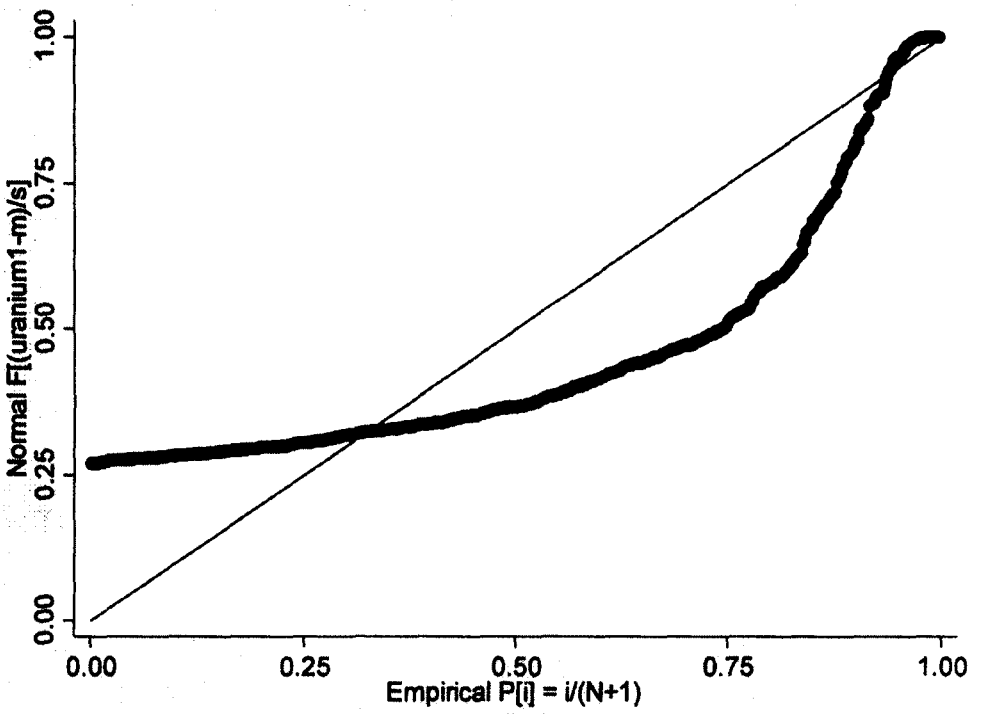
Models also adjusted for age, gender, BMI, employment status (current vs. former lead worker), enrollee status (phase I vs. II study entry), annual income (10, 10-20, 20-30, 30-40, and > 40 million won), education (< middle school graduate, < high school graduate, high school graduate, > high school), alcohol consumption (never, former, current), smoking dose [(cigarettes per day × years of smoking) in quartiles for current smokers and ex-smoker status], diastolic blood pressure, blood lead, tibia lead, and lead job duration (in antimony models only)

*p-value < 0.05; **p-value < 0.01; #p-value < 0.001

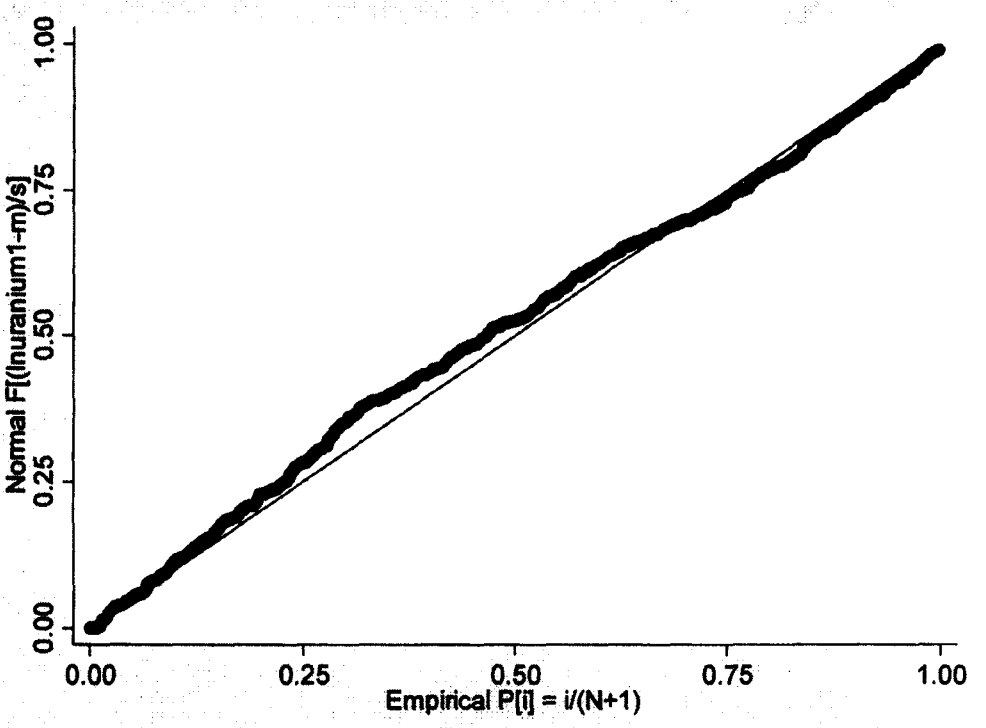
APPENDICES, CHAPTER 4

Appendix Figures 4-1 a-m. Urine Uranium Normal Probability Plots Pre and Post Log Transformation

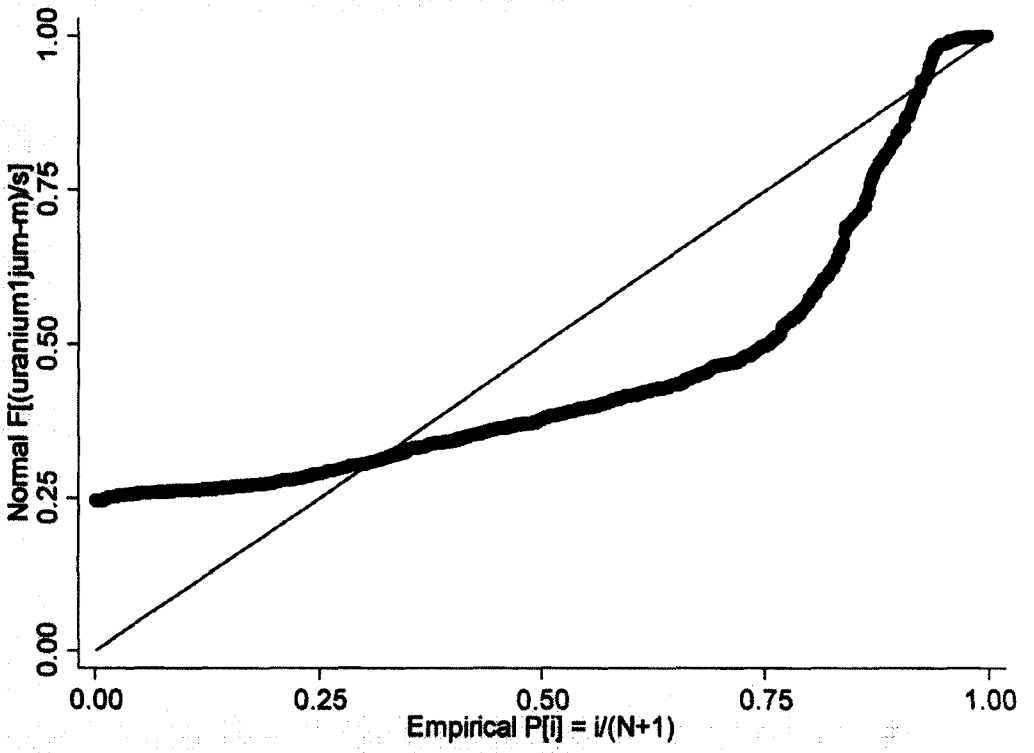
a. Urine Uranium (μg/L) Normal Probability Plot



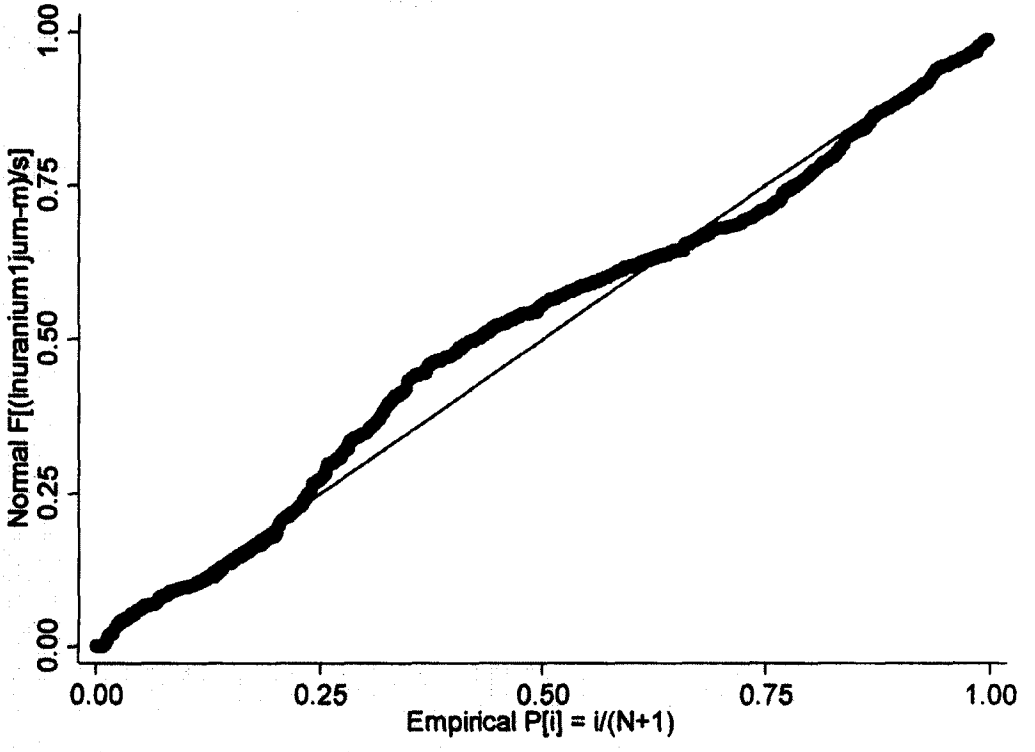
b. Ln-urine Uranium (μg/L) Normal Probability Plot



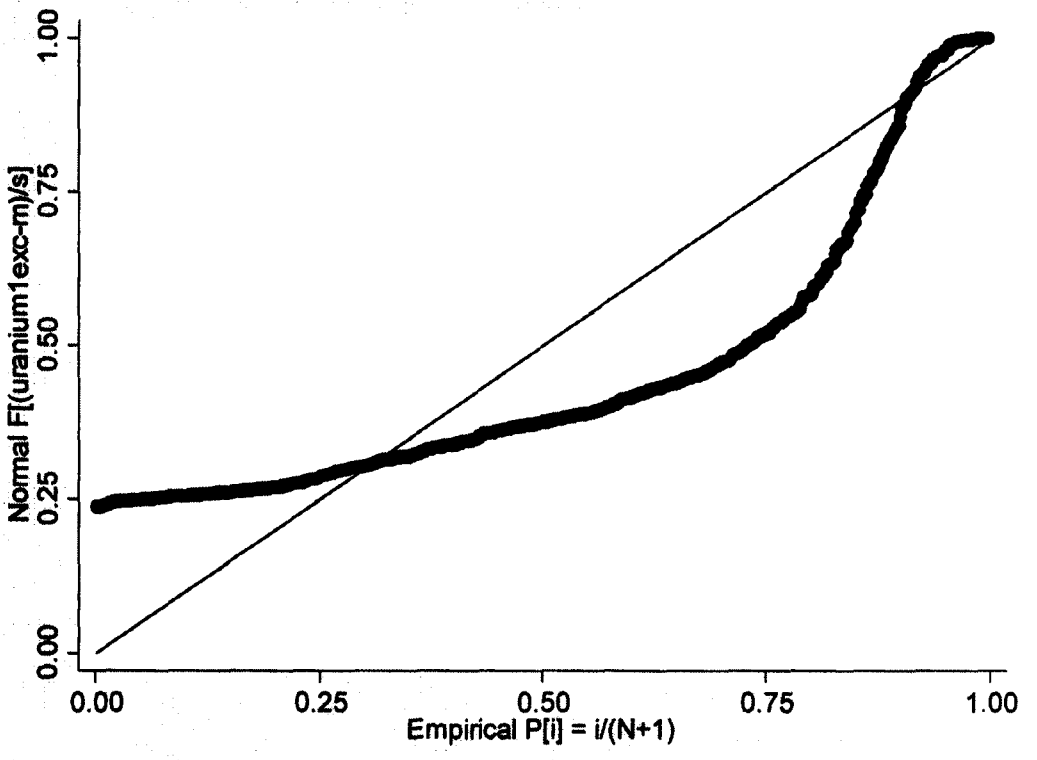
c. Urine Uranium (µg/g creatinine) Normal Probability Plot



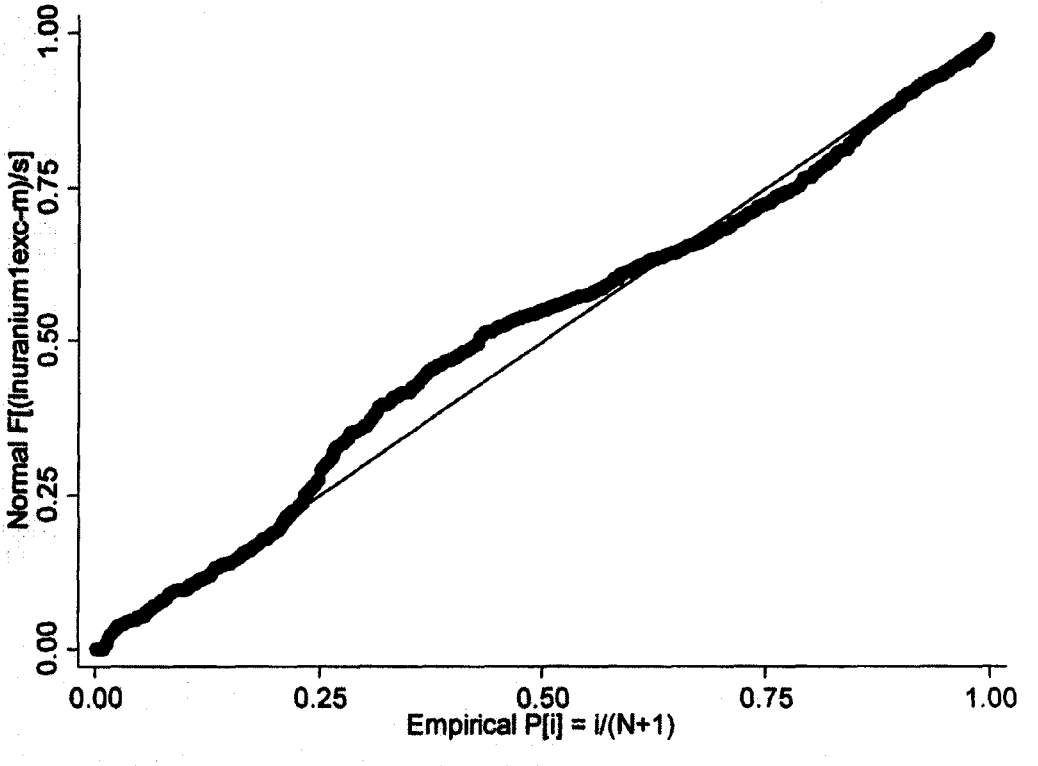
d. Ln-urine Uranium (µg/g creatinine) Normal Probability Plot



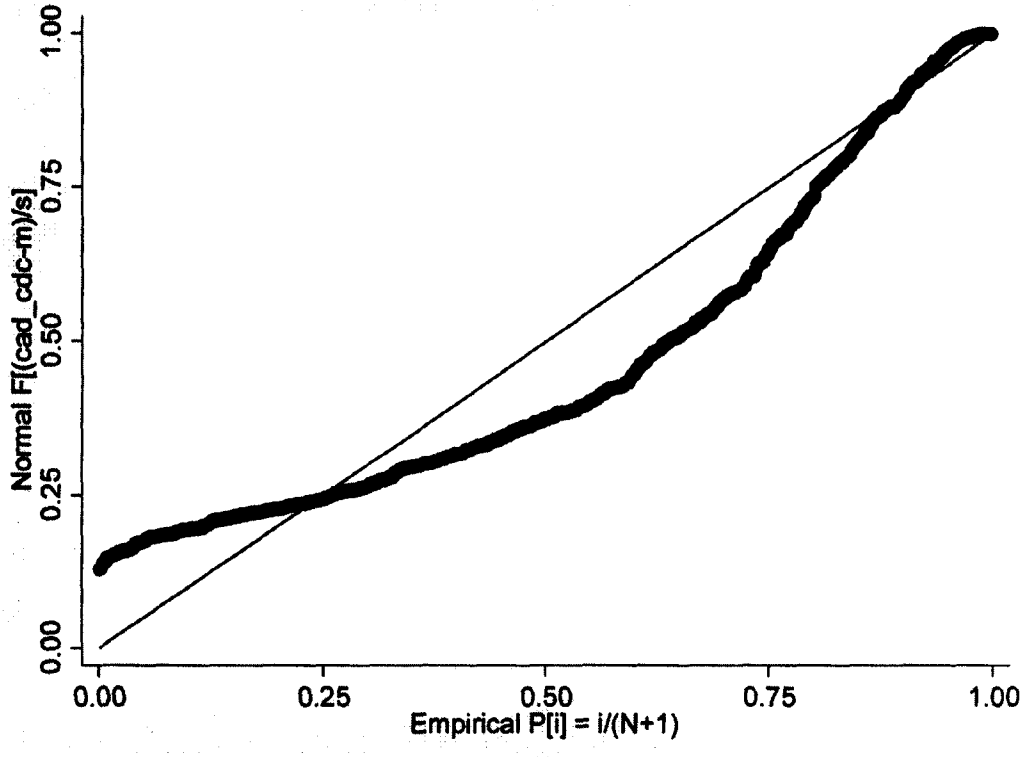
e. Total Urine Uranium excreted (μg)



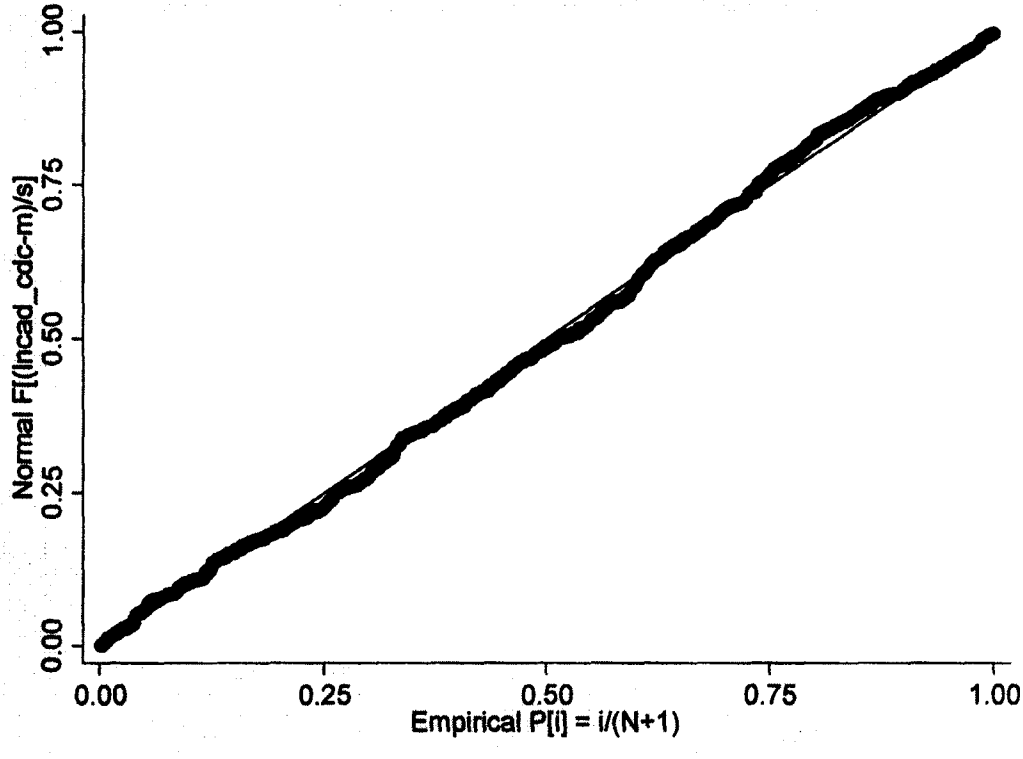
f. Total Ln-Urine Uranium excreted (μg)



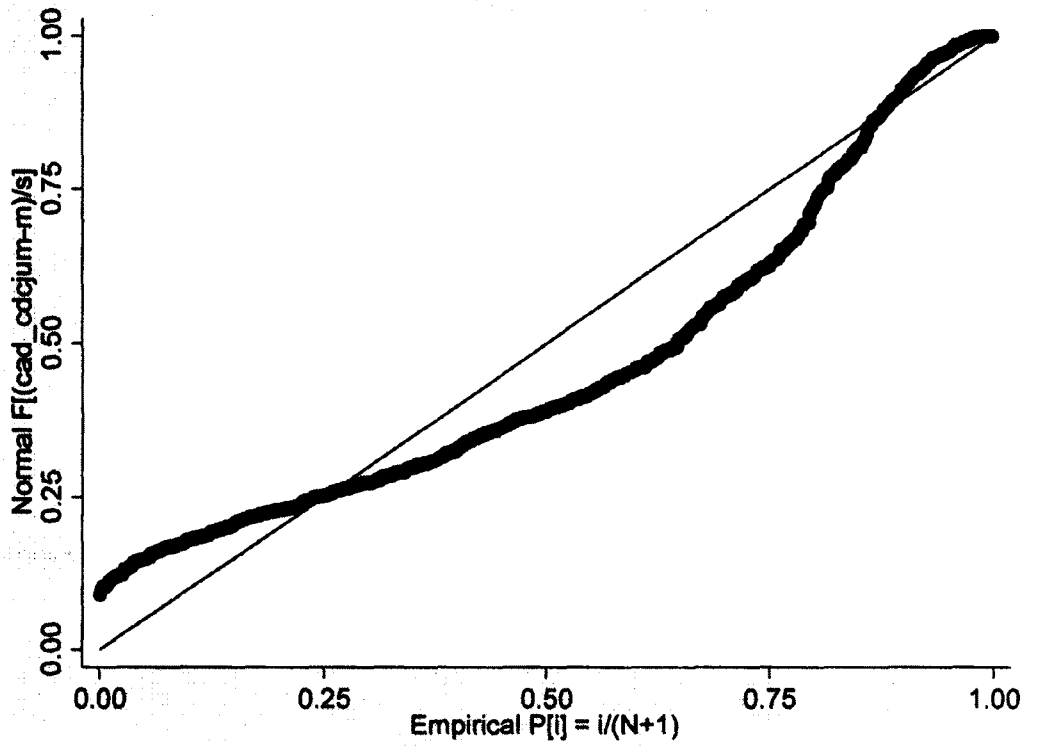
g. Urine Cadmium (µg/L) Normal Probability Plot



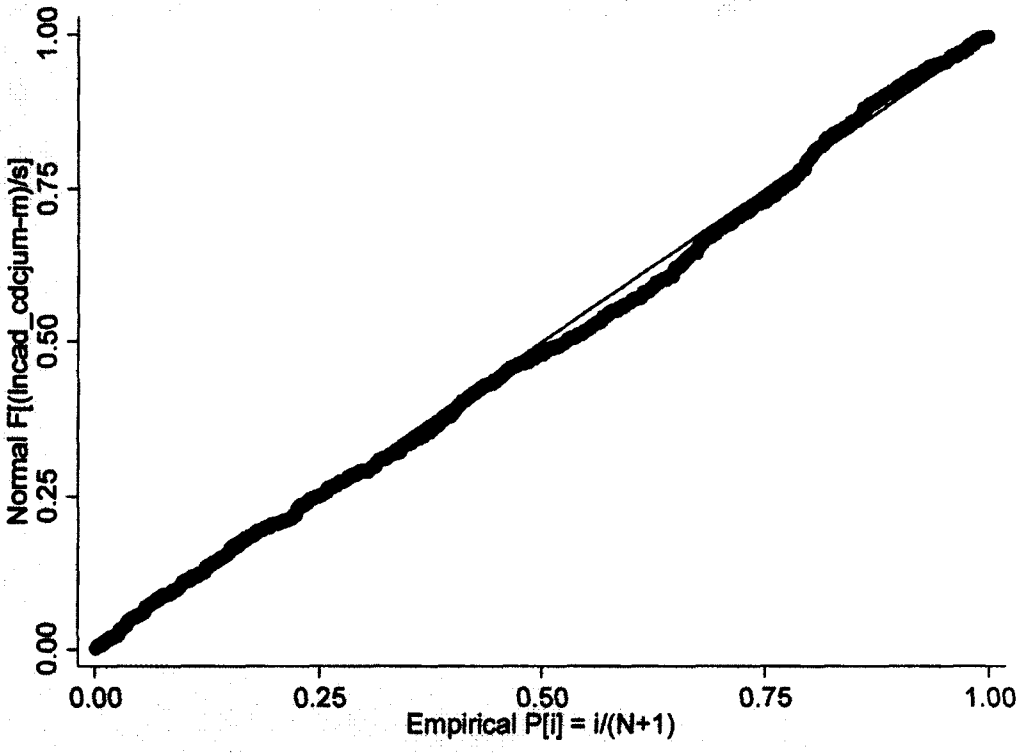
h. Ln-Urine Cadmium (µg/L) Normal Probability Plot



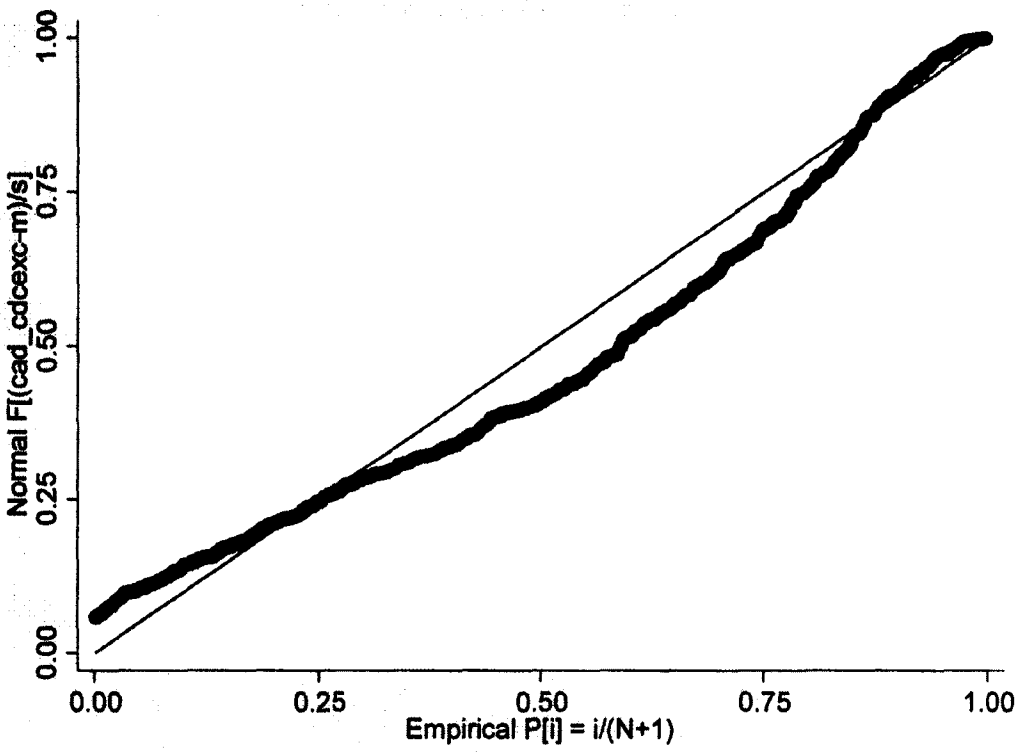
i. Urine Cadmium (µg/g creatinine) Normal Probability Plot



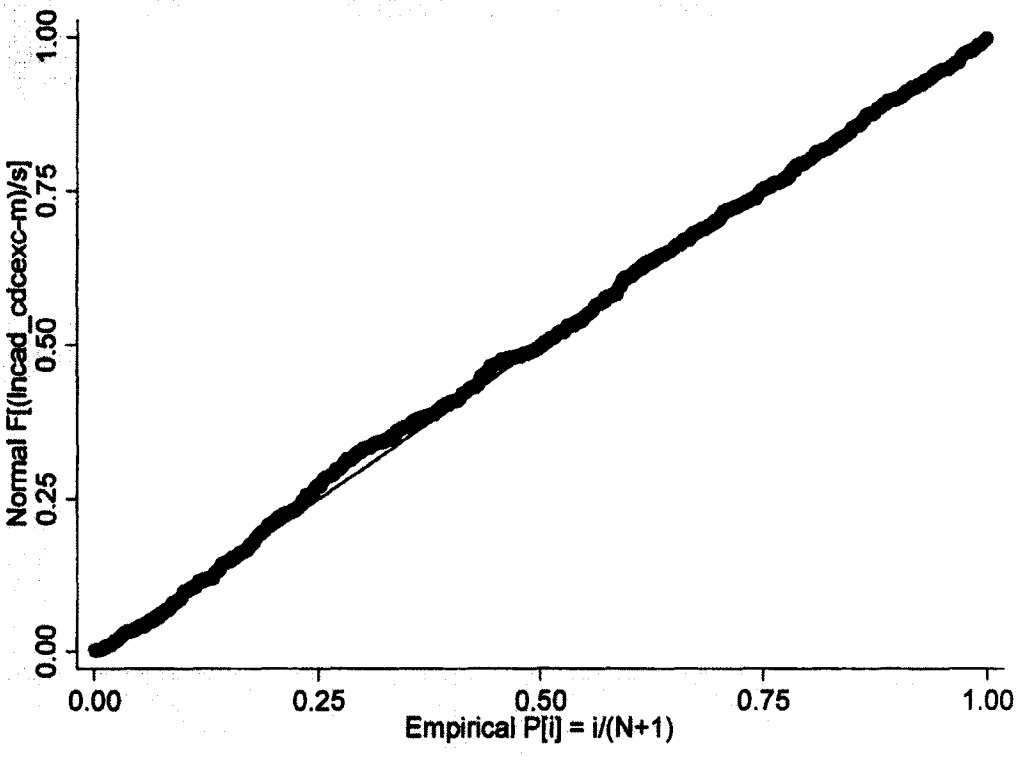
j. Ln-urine Cadmium (µg/g creatinine) Normal Probability Plot



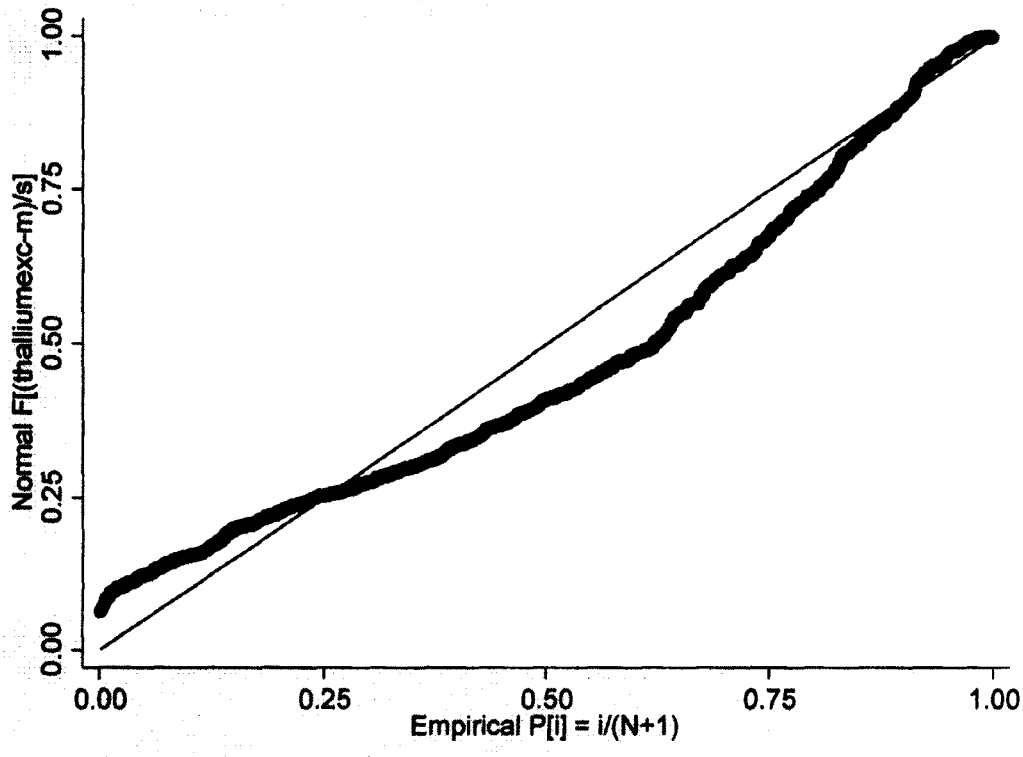
j. Total Urine Cadmium Excreted (μg)



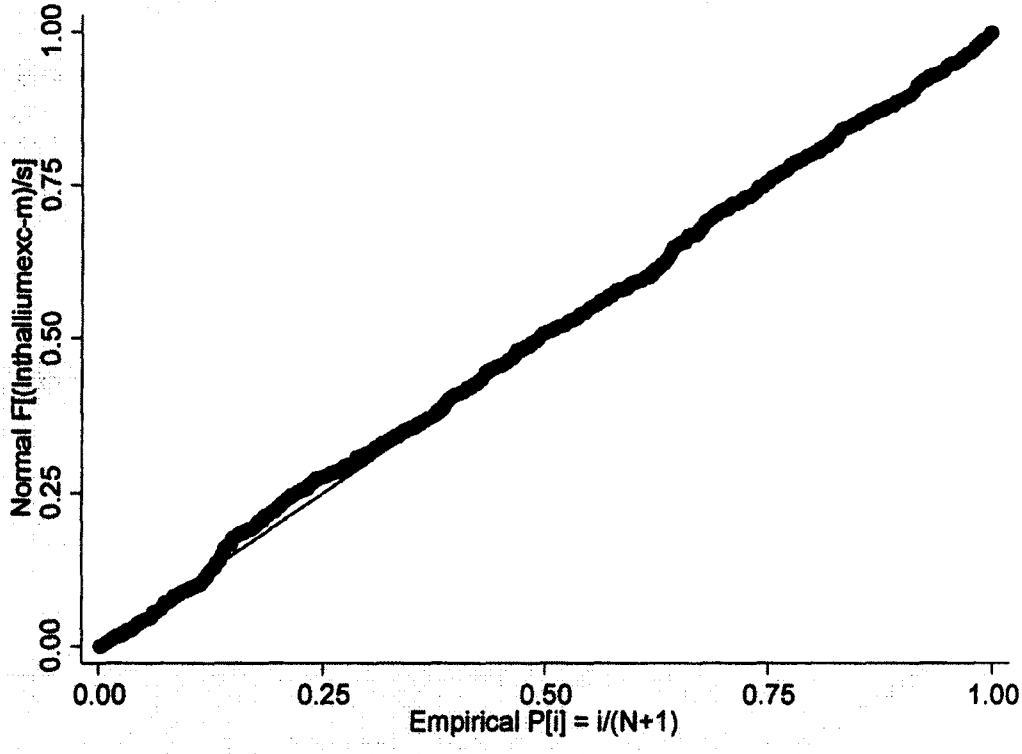
k. Total Ln-urine Cadmium Excreted (μg)



l. Total Urine Thallium Excreted (µg)



m. Total Ln-Urine Thallium Excreted (µg)



Appendix Table 4-1. A priori ln-urine uranium models and kidney outcomes by method of adjustment for urine dilution

Kidney Outcome	Model 1 Ln-Uranium µg/L[b] β coeff (95 % CI)	Model 2 Ln-Uranium µg/g creatinine β coeff (95 % CI)	Model 3 Ln-Uranium excreted β coeff (95 % CI)
MDRD eGFR, ml/min/1.73 m^2	-0.5 (-1.5, 0.5)	-0.5 (-1.5, 0.5)	-0.4 (-1.2, 0.4)
Cystatin-C-based eGFRm, ml/min/1.73 m^2	-0.6 (-1.4, 0.2)	-0.6 (-1.4, 0.2)	-0.7 (-1.5, 0.2)
Measured Creatinine Clearance, ml/min	-2.5 (-4.1, -0.9)**	-3.0 (-4.6, -1.5)#	0.2 (-1.3, 1.7)
Ln-NAG, µmol/h/g creatin	0.032 (0.001, 0.064)*	0.035 (0.004, 0.067)*	0.018 (-0.014, 0.051)

*p-value < 0.05; **p-value<0.01

[a]Models adjusted for age, gender, BMI, employment status (current vs. former lead worker), enrollee status (phase I vs. II study entry), annual income (10, 10-20, 20-30, 30-40, and > 40 million won), education (< middle school graduate, < high school graduate, high school graduate, > high school), alcohol consumption (never, former, current), smoking dose [(cigarettes per day × years of smoking) in quartiles for current smokers and ex-smoker status], diastolic blood pressure, blood lead, tibia lead and, ln-urine thallium, ln-cadmium and ln-urine creatinine (model 1 only)

CURRICULUM VITAE

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PERSONAL DATA

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EDUCATION/TRAINING

Expected	**Doctor of Philosophy Degree (PhD)** in Environmental Health Sciences, Johns Hopkins
May 2012	University Bloomberg School of Public Health, Baltimore, MD Thesis defense January 18, 2012: Multiple Metal Exposures and Kidney Outcomes in Lead Workers
2005	**Master of Science Degree (MS)** in Community and Public Health Nursing, Environmental Health Specialty, University of Maryland School of Nursing, Baltimore, MD
2001	**Bachelor of Science Degree (BS)**, major in Nursing, Binghamton University (State University of New York), Binghamton, NY
1998	**Associate in Applied Science Degree (AAS)**, major in Nursing, Broome Community College, Binghamton, NY

ADDITIONAL TRAINING

2007	Certificate in Risk Sciences and Public Policy, Johns Hopkins University Bloomberg School of Public Health
2005	Civil Mediation Training Course, Maryland Institute for the Continuing Professional Education of Lawyers, University of Baltimore, Baltimore, MD

PROFESSIONAL EXPERIENCE

2005-2010 **Environmental Health Liaison**, American Nurses Association (ANA) Department of Government Affairs, Silver Spring, MD
Identified state nurses associations with interest and capacity to undertake current environmental health initiatives (e.g. chemicals policy reform). Provided support to state nurses associations to promote legislative initiatives through such means as: talking points, letters to the editor; background information; suggested legislation; conference calls; and consultation with lobbyists. Developed and executed strategies to promote environmental health initiatives at the National Conference of State Legislators annual meeting(s). Represented the ANA in national environmental health coalitions and collaborative efforts with other national health and environment groups. Conducted environmental health focused presentations at professional nursing conferences and meetings. Educated state and federal lawmakers about ANA environmental health policies related to current legislation. Prepared quarterly reports to the ANA Board of Directors on federal and state legislative issues related to ANA environmental health policies.

2003-2005 **Graduate Assistant**, Center for Hazardous Substances in Urban Environments, University of Maryland School of Nursing/Johns Hopkins University, Baltimore, MD
Assisted in identification and assessment of communities with hazardous waste issues. Planned and implemented outreach and education activities with community, business, and government leadership within identified communities. Researched and collected data for scientific and community presentations.

2003-2005 **Community Health Nurse II**, Tuberculosis (TB) Control Program, Baltimore City Health Department, Baltimore, MD
Independently interviewed, assessed, and educated individuals (and families) with both active and latent TB. Conducted diagnostic tests and analyzed findings. Collaborated with program clinicians to determine and monitor treatment regimens. Coordinated, administered, and evaluated therapy for persons with latent and active TB.

2001-2003 **Public Health Nurse**, Tuberculosis Control, Broome County Health Department, Binghamton, NY
Performed duties as described for Community Health Nurse II (see above). Coordinated and conducted multiple outreach activities and community presentations targeting at-risk foreign born populations.

1998-2001 **Staff Nurse**, United Health Services Hospitals, Johnson City, NY
Served as rotating charge nurse for acute care hospital unit(s); assigned and delegated duties to registered nurses and certified nurses aides. Provided direct care to orthopedic, dialysis, and general medical-surgical patients.

Professional Research Presentations:

Associations of Multiple Metals with Kidney Outcomes in Lead Workers
Johns Hopkins School of Public Health Environmental Health Sciences Research Retreat
January 18, 2011
Baltimore, MD

Multiple Metal Exposures and Kidney Outcomes in Korean Lead Workers
International Society of Exposure Science Annual Conference
October 23-27, 2011
Baltimore, MD

Professional Poster presentations:

Associations of Multiple Metals with Kidney Outcomes in Lead Workers
Society of Toxicology Annual Meeting
March 6-10, 2011
Washington, DC

Impact of Multiple Metal Exposures on Kidney Outcomes in Lead Workers
World Congress of Nephrology
April 8-12, 2011
Vancouver, Canada

Professional Publications:

Shelley, R.L., et al., *Associations of Multiple Metals with Kidney Outcomes in Lead Workers.*, manuscript in prep., anticipated March 2012.

Shelley, R.L., et al., *Associations of Environmental Uranium Exposure and Kidney Outcomes in Lead Workers.*, manuscript in prep., anticipated March 2012.

Clouse, R., *Nursing organizations call for phase-out of agricultural practices that promote antibiotic resistance.* Policy Polit Nurs Pract, 2006. 7(1): p. 17-22.

Clouse, R., *Mercury use in health care: an occupational and public health hazard.* Am J Nurs, 2005. **105**(9): p. 104.

Special Skills:

Computing: STATA statistical software, Microsoft office products, Endnote reference software