



# **Final Scoping Study Report Epidemiology of Chronic Kidney Disease in Nicaragua**

**Prepared for the CAO-Convened Dialogue Process on Chronic Renal  
Insufficiency**

**Independent Report Prepared by Boston University School of Public Health  
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## Acronyms Used in Report

ACE	Angiotensin Converting Enzyme (as per “ACE Inhibitors”)
ACGIH	American Conference of Governmental Industrial Hygienists
ASOCHVIDA	Chichigalpa Association of Life
BEN	Balkan endemic nephropathy
BMI	Body mass index
BUSPH	Boston University School of Public Health
CAO	Office of the Compliance Advisor/ Ombudsman
CAS	Chemical Abstracts Service
CDC	Center for Disease Control and Prevention
CIDS	Centro de Investigación en Demografía y Salud (Center of Investigation of Demographics and Health)
CISTA	Centro de Investigación en Salud, Trabajo y Ambiente (Center of Health, Work and Environmental Investigation)
CKD	Chronic Kidney Disease
CR	Creatinine
CRD	Chronic Renal Disease
CRI	Chronic Renal Insufficiency
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
eGFR	estimated Glomerular Filtration Rate
EPA	Environmental Protection Agency
ESRD	End Stage Renal Disease
GFR	Glomerular Filtration Rate
GPS	Global Positioning System
HEODRA	Escuela Oscar Danilo Rosales Arguello hospital
HFRS	Hemorrhagic fever with renal syndrome
HTN	Hypertension
ICPMS	Inductively coupled plasma mass spectrometry
IDMS	Isotope dilution mass spectrometry
IFC	International Finance Corporation
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
INSS	National Institute of Social Security (Instituto Nacional de Seguridad Social)
IRB	Institutional Review Board
IRIS	Integrated Risk Information System
ISA	Ingenio San Antonio
LOD	Limits of detection
MCLs	Maximum Contaminant Levels

MDRD	Modification of Diet in Renal Disease (Study)
MINSA	Ministry of Health
MMWR	Morbidity Mortality Weekly Report
NAG	N-acetyl- $\beta$ -D-glucosaminidase
NE	Nephropathia epidemica
NHANES	U.S. National Health and Nutrition Examination Survey
NSAIDS	Non-steroidal anti-inflammatory drugs
NSEL	National Sugar Estates Limited ( <i>or Nicaragua Sugar Estates Limited- pg 155</i> )
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PKD	Polycystic kidney disease
RfD	Reference Dose
SAS	Statistical Analysis Software
SES	Socioeconomic Status
STD	Sexually Transmitted Disease
TLVs	Threshold limit values
TOR	Terms of Reference
UMOD	Uromodulin gene
UNAN-Leon	Universidad Nacional Autónoma de Nicaragua in Leon
USEPA	United States Environmental Protection Agency
USRDS	United States Renal Data System
UTI	Urinary tract infection
WBGT	Wet Bulb Globe Temperature

# EXECUTIVE SUMMARY

## I. Background

This document is a summary of a larger report that was produced as part of a contract issued to Boston University School of Public Health (BUSPH) by the Office of the Compliance Advisor/Ombudsman (CAO) of the International Finance Corporation (IFC) and Multilateral Investment Guarantee Agency (MIGA), World Bank Group. The report is a component of a process that was initiated by a complaint filed by the Center for International Environmental Law on behalf of the Chichigalpa Association for Life (ASOCHIVIDA), an organization of individuals who formerly worked at the Ingenio San Antonio (ISA), which is owned by National Sugar Estates Limited (NSEL). The complaint alleged that the IFC failed to address the health and well-being of workers or the environment when delivering a substantial loan to NSEL, the primary example of harm being an epidemic of chronic kidney disease (CKD), also referred to as chronic renal insufficiency (CRI).

In response to the initial complaint, CAO conducted a preliminary investigation and recommended that a dialogue process (hereinafter referred to as “Dialogue”) be initiated between representatives of ASOCHIVIDA and NSEL and convened by CAO. Early dialogue meetings led to preparation of Terms of Reference (TOR) for a Scoping Study to consider the following two questions: (1) What are the causes of CRI in the Western Zone of Nicaragua? and, (2) Is there any relationship between the practices of the ISA and the causes of CRI?

Boston University School of Public Health (BUSPH) was selected by the Dialogue participants to conduct the Scoping Study, and we assembled a team of researchers with expertise in epidemiology (Dr. Daniel Brooks and Dr. Ann Aschengrau), occupational and environmental health (Dr. Michael McClean and Dr. Madeleine Scammell), nephrology (Dr. James Kaufman and Dr. Daniel Weiner), and preventive medicine (Dr. Oriana Ramirez Rubio) to carry out the following tasks:

1. Review the existing information available on CKD in Nicaragua, identify data gaps, evaluate the feasibility and usefulness of additional studies, and identify study design options that could yield the necessary information;
2. Make fact-finding trips to Nicaragua to meet with Dialogue Table participants (ASOCHIVIDA and NSEL) and other key stakeholders (MINSAs, medical providers, researchers) for the purpose of gathering information;
3. Prepare a presentation of study design options and recommendations that is based on the information generated in Tasks 1 and 2 and best professional judgment;
4. Present and discuss the study design options and recommendations at a workshop with the Dialogue participants; and
5. Prepare a final report that proposes study activities that will contribute to answering the two causal questions posed by the Dialogue Table participants.

As described in Section V, the core team for the Scoping Study will be expanded to include additional investigators from Nicaragua and the U.S. to accomplish the planned activities.

Unlike many scholarly studies, we have approached this project more from the perspective of a response to a public health emergency rather than an academic research investigation. This project is being conducted as part of a carefully navigated process in which multiple stakeholders are engaged to answer practical questions of mutual concern. As such, an important component of this effort is to ensure that we address the issues that are most relevant to the participants of the dialogue process. We have not proposed a comprehensive, large-scale study that would be conducted over a relatively long period. We have instead proposed shorter-term discrete steps that have been designed to address key data gaps, the concerns of the dialogue participants, and, in our view, have the potential to yield high impact information.

### **What is Chronic Kidney Disease (CKD)?**

CKD is defined by either a reduced glomerular filtration rate (GFR) or by evidence of kidney damage. Early stages manifest with slight kidney damage that is commonly marked by albumin in the urine. Clinical symptoms often do not appear until later stages when GFR worsens. Research on the causes of CKD suggest that there are likely multiple factors involved at each stage, including susceptibility factors (which increase vulnerability to kidney damage), initiation factors (which cause kidney damage), and progression factors (which cause worsening damage) (Levey 2007).

### **CKD as global health problem**

Reflecting its rising incidence and prevalence, CKD is a major international public health concern. Its prevalence in developed nations such as the United States currently ranges from 13-16% (e.g. Coresh 2007, Zhang, 2008, Chadban, 2003) and likely reflects high rates of obesity. The major causes are diabetes and hypertension (Collins, 2009).

Less is known about the frequency of CKD in developing countries; however, screening studies have reported prevalences varying from 2 to 16% (e.g., Sumaili, 2009; Singh, 2009; Chen, 2009; Ito, 2008; Gutierrez-Padilla, 2009). Studies in developing countries also generally note a high prevalence of hypertension and diabetes in the affected population (e.g., Sumali, 2009; Singh, 2009), but diabetes and hypertension appear to be a less common cause of CKD in these countries.

Environmental toxins are also known causes of CKD that have often been linked to striking geographic variations in prevalence. Examples include the occurrence of nephropathy associated with ingestion of food contaminated with cadmium and mercury in Japan, ochratoxin A in Tunisia, and aristolochic acid in the Balkans (Jarup, 2002, Lesato, 1977, Abid, 2003, Bamias, 2008; Debelle, 2008).

CKD observed along the Pacific border of the Central American region – including Nicaragua – does not appear to correspond to the epidemiological patterns demonstrated in developed countries. Evidence suggests that CKD in Nicaragua affects a younger, predominantly male population at their most productive age. In spite of numerous previous investigations, there remain many unknowns with respect to etiology, risk factors, prevalence, and incidence in Nicaragua and elsewhere. Thus, it is imperative to extend and deepen our knowledge with the goal of developing prevention policies and practices that reduce the rate of this devastating disease.

## **II. Existing research on CKD in Nicaragua: evidence, interpretation and limitations**

### **Data on CKD in Nicaragua**

#### Mortality Statistics and Prevalence Studies

Available national mortality data from 1992 through 2005 indicate that the death rate due to CKD is much higher in Leon and Chinandega than other departments. In addition, these data show that mortality in the country as a whole has increased over time from approximately 4.5 per 100,000 inhabitants in 1992 to 10.9 per 100,000 in 2005; the greatest increases have occurred in Leon and Chinandega. The high mortality rates in Leon and Chinandega were seen in all age groups, including ages 15-49 years. Age-adjusted mortality rates were also much higher among men than women, particularly in these two departments.

Since 2003, several prevalence studies based on serum creatinine have been conducted primarily in Leon and Chinandega. These studies, which were based on random community samples and used estimated GFR (eGFR) as the measure of CKD, provide the most reliable data on prevalence of CKD (Torres 2007, Torres 2008a, Torres 2008b, Aragon 2009, Brookline 2008). Prevalence rates observed in these studies varied from 0-13.1% (median: 8.7%) and were above 8.0% in the sugarcane/banana and mining communities, Candelaria, La Isla, urban Chichigalpa, and Quezalguaque. Rates were lowest in the coffee and services communities. With the exception of northeast Leon, these studies found that men had a substantially higher prevalence rate than women, with ratios ranging from 3.1-38.1 and increasing to even higher ratios among more advanced cases.

Based on our review of mortality and prevalence data, we have drawn the following conclusions:

1. *The occurrence of CKD is higher in the departments of Leon and Chinandega compared to other areas of Nicaragua.* Mortality data provide strong evidence that CKD is more common in Leon and Chinandega than other areas of the country. While we cannot rule out the possibility that the observed elevation in mortality rates in Leon and

Chinandega is attributable to selection or information bias, it is difficult to imagine that these factors could explain such large excesses.

*2. The occurrence of CKD in the departments of Leon and Chinandega is higher among men than women.* The evidence is strongest that CKD is more common in men than women in Leon and Chinandega because it derives both from mortality data and prevalence studies. This fact alone is a powerful etiologic clue, because any identified cause(s) should be consistent with this observation.

*3. The occurrence of CKD is higher among younger age groups in the departments of Leon and Chinandega compared to other regions of Nicaragua and the U.S.* Age-specific mortality statistics and prevalence data from Quezalguaque, Candelaria, La Isla and Chichigalpa, as well as data collected by ASOCHIVIDA provide strong evidence that CKD is more common in younger residents of Leon and Chinandega than would be expected.

*4. The occurrence of CKD is elevated among certain occupational groups compared to the general population.* The five-community study in Leon and Chinandega conducted by UNAN-Leon CISTA shows a clear differentiation among men according to community, with the highest prevalence rates found in the two communities where sugar cane/banana cultivation and mining were the primary economic activity (Torres, 2007). The fishing community also had relatively high prevalence, while communities whose economies centered primarily on coffee and services had low rates.

Based on consideration of all the evidence, we believe the most appropriate interpretation of the data is:

- (1) There is a wide variation in the prevalence of CKD by occupational group in the region.
- (2) Sugar cane workers are one of the occupational groups with a high prevalence of CKD.
- (3) Sugar cane workers are not unique in having a high prevalence of CKD

These results do not necessarily mean that occupational exposures must be the cause of CKD. However, they do suggest that an occupational etiology -- either singly or contributory -- is a plausible hypothesis that needs to be addressed.

#### Prior Epidemiologic Research on CKD in Nicaragua

In addition to data on mortality and prevalence, 22 unique epidemiological studies that examined hypotheses about potential causes of CKD in Nicaragua were reviewed. These studies provide results on a wide variety of exposures, including certain occupations (generally defined as either agricultural or sugar cane work), heavy metals, and pesticides; medical conditions including dehydration, urinary tract infections, diabetes, and hypertension; use of non-steroidal anti-inflammatory drugs; lija and alcohol consumption; cigarette smoking, and family history of kidney disease. Taken together, these studies reported fairly consistent positive associations for (1) agricultural

work, (2) pesticide exposure, (3) dehydration, (4) hypertension, (5) lija consumption, and (6) family history of CKD. Results for the remaining exposures were either inconsistent or essentially null. However, due to their limitations (many of them unavoidable), most of these studies have better served as a preliminary stage of knowledge by screening hypotheses rather than testing them.

### **Limitations of current knowledge**

The validity of many of the studies was difficult to assess because we often did not have access to complete descriptions of the study methods. For example, it often was challenging to determine if bias was present or if confounding was adequately controlled. However, even among studies with sufficient description, we identified several limitations that make their interpretation problematic. These include uncontrolled confounding, recall bias from the use of interviews to collect retrospective exposure data, failure to consider synergistic effects of two or more risk factors, misclassification of exposure information, and low statistical power stemming from a small number of subjects.

One of the most concerning aspects of this body of research is uncontrolled confounding. Confounding means that the association is invalid because there is a mixing of effects between the exposure, the disease and a third extraneous variable known as a confounder. Evidence for uncontrolled confounding among the reviewed studies includes the failure to control any confounders in some studies, controlling for only a limited number of confounders in most others with little or no justification for controlling certain confounders while omitting others. For example, separate strong associations were reported for two related exposures --history of urinary tract infections and the use of NSAIDS; however, because analyses examining one exposure did not control for the other, it is impossible to determine if these associations are valid or if they confound one another.

Another important problem is recall bias, which stems from the use of interviews to collect retrospective exposure data. Recall bias occurs when there is a differential level of accuracy in the information provided by the compared groups (e.g. cases and controls). In this context, widespread awareness coupled with strong ideas about possible causes of CKD might lead to those already diagnosed with CKD (cases) reporting with a different level of accuracy than those not already diagnosed (controls).

Still another limitation is exposure misclassification, which is one of the most common problems in epidemiological research. This problem can arise when broad categories are used to classify exposure. For example, some studies defined the exposure as "agricultural work" or as "pesticide exposure," even though it is likely that only certain types or aspects of agricultural work and only certain types of pesticides increase the risk of CKD. While broad exposure classifications give a general idea of a putative cause, they make it difficult to identify effective preventive measures and tend to bias results towards the null (i.e., showing little or no association). Misclassification can also arise for "clinical" exposures. For example, true urinary tract infections are uncommon in

males, yet this condition was frequently reported among male subjects, particularly those with CKD.

Another drawback of the prior studies is their failure to simultaneously take into account the impact of two or more factors that may be working in concert to produce CKD, and which together may increase the risk of disease beyond what we would expect from simply adding the risks associated with each factor alone. For example, volume depletion may make the kidneys more susceptible to the effects of other exposures such as heavy metals and NSAID use. While each factor alone may lead to a modest increase in risk, the combination of both factors may lead to a large increase in risk.

Last but not least, none of the existing studies test other hypotheses regarding the causes of CKD in Nicaragua, including exposure to aristolochic acid (known to cause CKD in the Balkans); known infectious diseases; and the use of nephrotoxic antibiotics and other drugs.

In summary, the 22 epidemiologic studies provide results on a wide range of hypothesized causes of CKD. Taken together, these studies reported fairly consistent positive associations for agricultural work, pesticide exposure, dehydration, hypertension, lija consumption, and family history of CKD. Positive associations were observed for these six exposures even among the few studies that controlled for confounding variables. Results for the remaining exposures were either inconsistent or essentially null. Because the positive findings were relatively consistent and some confounders were controlled, we have slightly more confidence in their validity. However, as noted above, all of the prior studies were questionnaire-based, and so we cannot rule out the possibility that recall bias (as well as other problems) accounts for the findings. Thus, as described in greater detail in Section IV, we recommend that an entirely different approach be taken for future studies of CKD in Nicaragua. Instead of relying solely on questionnaires, our recommended approach includes environmental sampling, analysis of biological samples, work observation, and a record-based cohort study, among other activities.

### **III. Areas of potential investigation**

Although the data summarized in Section II provide important clues, it is our view that there is insufficient evidence to draw any conclusions about the cause(s) of the elevated rates of CKD.

Although there may be a single etiologic agent responsible for the excess occurrence of CKD in Nicaragua, it is also quite possible that there is no single cause of CKD but rather a combination of factors that increase susceptibility, lead to initiation, and/or hasten progression. Therefore, possible causes need to be considered not only individually but as potentially acting together. As one hypothetical example, an occupational or environmental exposure might increase the risk of CKD only in the

presence of an infectious disease. This notion of multiple, or synergistic, factors complicates the effort to clearly identify the reasons behind the epidemic of CKD.

Based on our review of the studies described above, our literature review, and our discussions with nephrologists, epidemiologists, environmental and occupational health experts, ASOCHIVIDA, NSEL, and the CAO, we have summarized 17 areas of potential investigation. The table below describes these hypotheses and provides our evaluation of the implications for our future work. Please consult the full report for references.

<b>Areas of potential investigation</b>	<b>Implications for Action</b>
<p><u>Agrichemicals:</u> Agricultural chemicals include a variety of synthetic compounds, often used in combination at different times during the season depending on the target pest and the crop. The regions of Chinandega and Leon are currently areas of high sugar cane production and historically were areas of high cotton production. There are concerns among workers that exposure to agrichemicals is a cause of CKD.</p>	<p>The main evidence in favor of the agrichemical hypothesis is the highly probable exposure to chemicals among workers. While the association between agrichemicals and CKD is unknown, agrichemical exposure is associated with a range of other health effects. We are treating this as a high-priority hypothesis, and we will examine this hypothesis through environmental sampling, occupational record review, and possibly biological sampling.</p>
<p><u>Volume depletion:</u> Although volume depletion is not a recognized cause of CKD, it is recognized to predispose to acute kidney injury. In fact, the use of prophylactic volume expansion is the cornerstone for the prevention of acute kidney injury after the administration of nephrotoxic agents.</p>	<p>Volume depletion likely is a common occurrence in any population of workers exposed to the combination of high environmental temperatures and strenuous physical exertion. We will examine this hypothesis through work observation and occupational and medical record review.</p>
<p><u>Muscle damage</u> Muscle damage (rhabdomyolysis) is a well-recognized cause of acute renal failure. It is not typically considered to be a cause of CKD, although acute renal failure is associated with subsequent CKD. Additionally, there are isolated reports of chronic interstitial nephritis as a consequence of rhabdomyolysis.</p>	<p>Rhabdomyolysis is a recognized, if rare, cause of acute kidney injury and may occur with exertional heat stroke. With increasing recognition that episodes of subclinical renal injury may lead to CKD and the possibility that repeated muscle breakdown may be occurring in cane workers, this etiology deserves specific attention. We will examine this hypothesis through work observation and occupational and medical record review.</p>

<p><u>Systemic Infections:</u> Many infections are associated with both environment (reflecting poor sanitation and hygiene conditions) and occupational exposures. Infectious diseases such as leptospirosis, hantavirus or malaria are known to cause acute renal failure. There is limited evidence related to their role as causative agents of CKD. However, infectious disease processes may work as precursors or synergistically with other nephrotoxic insults.</p>	<p>This hypothesis is difficult to study. We will explore the possibility of using biological samples to test for the presence of leptospirosis IgG, and will also use existing records such as pre-employment screening questionnaires and from medical records. However, the reliability of these sources is likely not very high and the yield is likely to be low.</p>
<p><u>Heavy Metals</u> Chronic exposure to heavy metals, most notably lead and cadmium, is associated with chronic tubulointerstitial nephritis.</p>	<p>We know little about the sources, distribution, and levels of lead or cadmium exposure in Nicaragua. Potential sources include occupational exposure, lead-containing products (e.g., paint), and emissions from volcanoes, which are present and active in the region. We will examine this hypothesis by environmental sampling of soil, water, and food, and by biological sampling.</p>
<p><u>Uranium</u> Animal studies, as well as studies of occupationally exposed persons, have shown that the major health effect of uranium is chemical kidney toxicity, rather than a radiation hazard.</p>	<p>The main potential source of exposure to uranium in northwestern Nicaragua is likely volcanic emissions. We will examine this hypothesis through environmental testing of soil, water, and food.</p>
<p><u>Aristolochic Acid</u> Aristolochic acid obtained from seeds of the common plant, <i>Aristolochia</i>, is a well-known nephrotoxin and has been incriminated as the source of several epidemics of CKD. Cases of chronic interstitial nephritis have been linked to herbal remedies containing aristolochic acid and bread made from wheat contaminated with the seeds of <i>Aristolochia clematidis</i>.</p>	<p>Because of its established nature as a nephrotoxin, aristolochic acid should be investigated. <i>Aristolochia</i> is common in Nicaragua, and species of <i>Aristolochia</i> used for herbal medicinal purposes (eg, snakebite) may have nephrotoxicity. The focus will be on identifying plants used for herbal remedies and examination for any possible contamination of food supplies.</p>
<p><u>Medications</u> Medications are a common cause of acute kidney injury and may be associated with CKD. One of the classic epidemics of CKD was associated with use of analgesics containing phenacetin. Use of common non-steroidal anti-inflammatory drugs (NSAIDs), including ibuprofen, naproxen and diclofenac, all of which are used widely in Nicaragua, has been associated with CKD. Certain antibiotics also have kidney toxicity. Kidney failure associated exclusively with NSAIDs is unusual; rather NSAIDs are more often a cause of acute renal</p>	<p>Because of their common use and established nature as nephrotoxins, NSAID use, combination analgesic use and aminoglycoside use, as well as the use of traditional herbal remedies, will be investigated through qualitative interviews and medical record review.</p>

<p>failure in the setting of severe volume depletion or other nephrotoxins.</p>	
<p><u>General Alcohol Consumption</u>  In numerous epidemiologic studies in the US and elsewhere, alcohol has not been associated with development or progression of chronic kidney disease. Excessive alcohol consumption is associated with hyperuricemia, and there is suggestive evidence that hyperuricemia may adversely affect kidney function. Alcohol also exerts a diuretic effect and may exacerbate volume depletion.</p>	<p>The main evidence in favor of the alcohol hypothesis is the presumed increased consumption among men and its association with CKD in a number of studies. This hypothesis is difficult to study other than by questionnaire. While we will collect data as the opportunity presents, we are not treating this as a high-priority hypothesis.</p>
<p><u>Guaro lija</u>  Guaro lija (or simply “lija”) is a form of rum that is produced at a commercial distillery, presumably under appropriate and safe conditions, and then is shipped in bulk to small independent distributors and retailers where it is further processed and then sold in plastic bags to individual consumers. At the time of production, the rum is the same as that which is eventually sold in bottles but has a much higher concentration of ethanol (95%). “Lija” should not be confused with homemade alcohol. It has been suggested that lija has an independent association with incident CKD other than simply being a form of alcohol, possibly due to the introduction of an unknown toxin somewhere in the chain between production at the factory and consumption by the individual.</p>	<p>The main evidence in favor of the lija hypothesis is the presumed increased consumption among men and its repeated strong association with CKD in a number of studies. However, because of the difficulties in identifying a contaminant which is likely to have been present only historically and sporadically, it may be difficult to make much progress in investigating the potential role of lija consumption. It will be helpful to better understand past and present practices regarding lija manufacture, distribution, sale, and consumption, as well as the potential value of testing of current samples. We will begin by conducting key informant interviews and then assess whether there is a basis for further study.</p>
<p><u>Kidney stones</u>  Nephrolithiasis is a recognized cause of kidney failure. It is known that stones occur more commonly in people who work at high environmental temperatures. Therefore, there may well be an increased risk for kidney stones and possibly related CKD in Nicaragua.</p>	<p>Although risk factors for stone disease are prevalent in the population of interest, kidney stones are considered a rare cause of CKD. However, given the ease of identification from existing ultrasounds and the reported high prevalence among persons with CKD, we will investigate this hypothesis using medical records.</p>
<p><u>Structural kidney disease</u>  Structural kidney disease encompasses a broad group of kidney diseases, both congenital and acquired, which are usually easily recognized with renal imaging using either ultrasound or computed tomography.</p>	<p>Structural kidney disease is unlikely to account for the increased prevalence of CKD in Nicaragua, but prevalence can be easily ascertained from a review of existing renal ultrasounds in medical records.</p>
<p><u>Diabetes</u>  Diabetes is a major cause of CKD worldwide, particularly in the developed world because of the worsening obesity epidemic. Diabetic kidney disease eventually develops in 25-50% of patients with diabetes, although the majority</p>	<p>Based on available data, diabetes does not appear to be the major cause of CKD in sugar cane workers. However, because diabetes is a well-known and common cause of CKD, its prevalence in the affected population will be estimated. We will explore</p>

<p>of these individuals do not develop kidney failure. The generally low prevalence of diabetes, even among people with CKD in Nicaragua, suggests that, while diabetes is an important risk factor for developing CKD on an individual level, it likely accounts for only a small portion of the excess occurrence of CKD at the population level. No clear gender specific risk has been identified.</p>	<p>this hypothesis through review of medical records.</p>
<p><u>Hypertension</u> Because hypertension is highly prevalent in developed countries, it is an important cause of CKD accounting for 25-40% of cases of kidney failure. However, hypertension also occurs as a complication of CKD. Approximately 80-85% of patients with CKD will have hypertension and the prevalence of hypertension increases as severity of CKD increases.</p>	<p>None of the studies that have measured hypertension have been able to distinguish between hypertension that occurred prior to CKD or as a complication after CKD diagnosis. Medical records will be reviewed in an attempt to examine hypertension during a time period prior to the onset of CKD.</p>
<p><u>Glomerulonephritis</u> Glomerulonephritis is a common cause of CKD worldwide and, in some countries, such as Japan, where IgA nephropathy is highly prevalent, glomerulonephritis is the leading cause of CKD. Some forms of glomerulonephritis appear to have an increased incidence in certain geographic areas.</p>	<p>It is unlikely that glomerulonephritis is an important contributor to the increased prevalence of CKD in the study population. We will review medical records for evidence of the presence of high-grade proteinuria and hematuria, which provides a simple means of estimating the prevalence.</p>
<p><u>Urinary Tract Infection (UTI)</u> In multiple studies conducted in Nicaragua, UTI has been associated with prevalent CKD. Abnormal ureteral implantation is the most common urologic anomaly in children and may predispose to recurrent UTIs. Severe pyelonephritis and recurrent UTIs have been associated with subsequent renal scarring, but this is an unusual cause of kidney failure in adults, and particularly in men.</p>	<p>The prevalence of UTIs could be addressed in a study of children where there is less recall bias; while this would provide important information, it does not directly address the relationship between UTIs and CKD among adult workers. An important question among adults is the practice of medication administration among clinicians for flank pain and/or dysuria, as well as the diagnoses associated with these symptoms (UTI, musculoskeletal pain, sexually transmitted diseases, etc.) We will begin to address this hypothesis through qualitative interviews among clinicians.</p>
<p><u>Genetics and CKD</u> Multiple lines of evidence suggest that the susceptibility to develop CKD may have a significant genetic component. There are several kidney diseases that are caused by mutations to a single gene. In addition, there is now extensive evidence that suggests that susceptibility to kidney disease in the general population has a genetic component.</p>	<p>A monogenic etiology for the prevalent CKD in the population is highly unlikely. Although there may well be genetic susceptibility factors, identifying such factors is costly, difficult, and unlikely to have an immediate impact on the at-risk population. No genetic analytic component is planned in the short term. However, we will consider storing samples for subsequent genetic testing.</p>

## **IV. RECOMMENDED ACTIVITIES**

### **Introduction**

In the previous section, we summarized several hypotheses based on knowledge about general causes of CKD as well as less well-established factors that might be operating in Nicaragua. This section proposes a set of nine activities that at least touch on the entire range of hypotheses, while at the same time focusing primary attention and resources on those areas that we have deemed to be highest priority. To appreciate the rationale for selecting these activities, it is important to return to the original mandate from the Dialogue process, which was to recommend activities that could 1) identify the causes of CRI in the Western Zone of Nicaragua, and 2) evaluate the relationship between the practices of the ISA and the causes of CRI. It is also important to consider additional information that has been emphasized during the Dialogue process:

1. the question of the company role is critical to making progress among the parties;
2. the timeframe must be as short as possible consistent with the requirements of good scientific methodology; and
3. resources are finite.

For these reasons, our recommended activities are primarily aimed at answering the question related to occupational practices. However, a number of the recommended activities are not directly related to occupational exposure. These are included to evaluate established causes of CKD that have never been assessed in the region and to address the possibility that both occupational and non-occupational factors are interacting in a synergistic manner to greatly increase the risk of CKD.

For a number of reasons, we have not prioritized the conduct of another prevalence study at this time. First, neither the costs nor time involved in mounting such a study are trivial. Second, a number of studies have already been conducted and it is unlikely that a new study would result in substantially different conclusions. Third, other groups are currently conducting or just beginning studies that can provide comparable information. For example, UNAN-CIDS in collaboration with the University of North Carolina is currently collecting data from approximately 3,000 households in the municipality of Leon. We will monitor this and other prevalence studies for significant results and incorporate evidence from these studies into our final assessment. The primary gap in knowledge that we can address is examining the potential effect of occupational, environmental, behavioral, and medical exposures more thoroughly than other groups have been able to accomplish to date.

In carrying out the recommendations described below, we will need to maintain flexibility since early findings may lead us in new directions. We will also need to have frequent and open communication with the Dialogue parties, as well as collaboration with MINSA

since this is the agency that has overall responsibility for the health of the Nicaraguan people.

Another key element of a successful study will be to solicit and receive input from other scientists. There is too much at stake in this study for our plans and activities not to be scrutinized by outside reviewers. The typical mechanism for providing this input in a research study is a Scientific Advisory Board (SAB), which is composed of a group of researchers chosen for their expertise in different areas relevant to the study. Preliminarily, we propose a SAB comprised of four members, with at least two from Nicaragua or elsewhere in Central America, which would meet two times a year. The SAB would review study designs and protocols, and substantive changes in designs and protocols, as well as review issues related to implementation such as recruitment; data collection, processing, and analysis; sample collection, storage, processing, and analysis; and ethical concerns.

## **Specific Recommendations**

We recognize that obtaining agreement on the final details of all study design elements (i.e. sample locations, etc) prior to implementation is an essential component of our effort if we expect all stakeholders to accept the eventual findings. Accordingly, all design elements will be discussed with representatives of NSEL and ASOCHIVIDA prior to implementation, and a representative from each group will be invited to accompany the field team. Each recommendation is described in one of the subsections below.

### 1. Environmental Sampling

In the northwestern region of Nicaragua, the extent to which surface soil may be contaminated with metals or agrichemicals has not yet been explored while comparable investigations of drinking water have been limited. Similarly, the presence of metals or aristolochic acid in food has not yet been investigated. We therefore propose to collect samples of surface soil, drinking water, and food, and analyze these samples for agrichemicals, metals, and aristolochic acid as appropriate.

Samples will be collected from 5 categories of agricultural fields, which include:

- ◆ fields at the ISA that have never been used for crops other than sugarcane
- ◆ fields at the ISA that are currently used for sugarcane but previously used for other crops
- ◆ fields owned by private landowners but leased and operated by NSEL for the production of sugarcane
- ◆ fields neither owned nor operated by NSEL and that are used for the production of sugarcane
- ◆ fields neither owned nor operated by NSEL and that are used for the production of crops other than sugarcane

Samples will also be collected from five residential communities that differ according to primary industry of employment, which would be expected to be associated with different prevalences of CKD. We plan to include La Isla and Candelaria because current and former workers at ISA and their families comprise the majority of residents. The remaining three communities have yet to be selected but would differ in primary industry and include few or no ISA workers.

First, we propose to review the toxicology and physical/chemical properties of a set of agrichemicals mutually agreed upon by NSEL and ASOCHIVIDA for the purpose of finalizing an appropriate list of analytes for each sample type, prior to conducting the very expensive analysis of agrichemicals in environmental samples.

Second, we propose to collect approximately 200 surface soil samples from the ten areas listed above. The sampling locations within each area will be selected to be representative of each field or community. A portion of each sample will be analyzed for metals while a portion will be stored for possible future analysis of agrichemicals. Linear regression models will be used to determine whether contaminant levels are significantly different by microenvironment within each area. Since CKD prevalence data are available for each of the residential communities, we will investigate whether contaminant levels by community are consistent with the observed differences in CKD prevalence. Additionally, an analysis of potential health risks associated with contaminants in surface soil will be conducted in accordance with the USEPA's risk assessment guidance for human health evaluations.

Third, we propose to collect a maximum of 200 drinking water samples from the same general locations as the surface soil samples. The sampling locations will be selected to characterize the primary sources of drinking water within each field category and from the same residences where surface soil samples were collected. An aliquot of each sample will be analyzed for metals and a second will be stored for possible future analysis of agrichemicals. The analytic approach for analysis of water samples will be the same as for soil samples. In addition, the levels of contaminants in drinking water will be compared to USEPA Maximum Contaminant Levels.

Fourth, we propose to evaluate the extent to which food may be contaminated with metals or aristolochic acid. We propose to administer a short dietary survey to the 20 residents in each the five communities who participate in the surface soil and drinking water investigation (total of 100 surveys). The purpose of the survey will be to assess the types and sources of commonly consumed food so that food samples can be collected and analyzed for metals and aristolochic acid. For metals, the samples will be homogenized, extracted, and analyzed; however, the analysis of aristolochic acid is not a common analytical procedure with standardized protocols. Therefore, we will first evaluate the feasibility, logistics, and costs of analysis. The data analytic approach will be analogous to those employed for soil and water.

Possible challenges: The environmental sampling has been proposed and designed as a screening level effort given that there has been limited previous environmental

sampling in the region. However, exposures to metals and agrichemicals likely occurred over many years and over a large geographic area, whereas our proposed investigation will focus on current conditions using samples collected from a relatively small area. Accordingly, there is the potential for findings from this activity to have a high impact if levels are elevated or if clear patterns are observed; however, the lack or elevated levels or the lack of clear patterns would need to be interpreted cautiously. Given the limited scope of the assessment, there could still be elevated levels that are missed because they are present in different areas or because they occurred at an earlier time and are no longer present.

## 2. Biological Sampling

Partly based on the results of the environmental sampling, we will consider the benefits of analyzing biological samples (potentially including blood, urine, hair, nails, and bone x-rays) for metals, selected agrichemicals, and aristolochic acid. One potential source of samples is current workers at ISA, all of whom have routine blood and urine testing every year. A possible second source could be a random sample of the five communities in which the environmental sampling will be conducted.

Within each of these five communities, we will select two adult family members who reside in the same homes, such that there will be 20 matched pairs from the two sugarcane communities and 10 matched pairs from each of the other communities. We will also administer a questionnaire to determine whether metal, agrichemical, or aristolochic acid levels differ significantly by work history or by sex. We will also assess the association between these levels and CKD.

Possible challenges: Similar to the environmental sampling, the biological sampling has been designed as a screening level effort. Biomarker levels integrate exposure across all exposure routes and pathways and could potentially yield information that would be missed if we relied on environmental samples alone. However, the half-life of metals in biological samples is shorter than in environmental media and we will be analyzing samples collected from a small subset of the population at a single point in time. Accordingly, there is the potential for findings from this activity to have a high impact if levels are elevated or if clear patterns are observed; however, the lack or elevated levels or the lack of clear patterns would need to be interpreted cautiously. Given the limited scope of the assessment, there could still be elevated levels that are missed because they are present in different subsets of the population or because they occurred at an earlier time but were no longer present in blood when samples were collected.

## 3. Work Observations

The work observation study proposes to address two hypotheses: volume depletion and muscle damage. Major risk factors for volume depletion and muscle damage among sugarcane workers include: ambient temperature and humidity, work effort, hydration status at the start of the work day, ability of the kidney to regulate perfusion at extremes

of volume, alcohol consumption, and use of medication (NSAIDS, others). Accordingly, we will study workers in three occupational groups: (1) sugar cane harvesters; (2) cutters, seeders, and weeders; and (3) a “control” group of ISA factory workers. We plan on studying 25 workers from each of the three groups, with repeated measurements from each worker over three workdays. Blood samples will be measured for creatinine, creatine kinase, and myoglobin, and urine samples will be measured for specific gravity, myoglobin, albuminuria, and tubular proteinuria. Questionnaires, a physical examination, and work observations will provide additional information. We will also assess the industrial hygiene practices and health and safety program at ISA, such as schedules, conditions, activities, and personal protective equipment.

Estimates of volume status (e.g.: weight change, change in serum creatinine, etc) and estimates of muscle damage (e.g.: change in serum creatine kinase and serum myoglobin) will be the focus of comparison among the three worker groups. If we find that volume depletion and muscle damage are occurring, analyses will be performed to look for possible risk factors, such as age, environmental temperature, work effort, hydration, thirst, and recent medication use.

Possible challenges: It will be important to ensure that the working conditions during the observation study are representative of typical current and historical work practices, in terms of environmental conditions and work intensity. Similarly, the workers studied also need to be representative of those at risk, specifically their physical conditioning and work effort should be within the range of a typical worker. To ensure to the extent possible that these requirements are met will require cooperation from a number of stakeholders, including representatives from NSEL, ASOCHIVIDA, unions, and other retired workers (who are not associated with ASOCHIVIDA). Even with these precautions, it is possible that because the workers are being observed their behavior, particularly their fluid intake, may be better than usual practice. As such, we expect that data may represent practices somewhat better than typical. Therefore, the absence of signs of volume depletion or muscle damage will not absolutely exclude these factors as important in the development of CKD in the population at risk.

The markers chosen for muscle damage, creatine kinase and myoglobin, are relatively sensitive but may not detect all instances of subclinical muscle damage. Nevertheless, in a previous study of exercise in normal volunteers these markers were able to detect a remarkably high incidence of muscle damage. The use of markers of tubular proteinuria to detect kidney damage should be considered exploratory, since there are no preliminary data establishing their utility in patients with volume depletion or myoglobinuria. The absence of positive findings would not definitively exclude the possibility of subclinical renal damage, but alternative measures such as inulin clearance or histologic examination are not practical.

#### 4. Cohort Study of ISA Employees

We recommend assembling data from past and present NSEL workers in order to determine the association between occupational characteristics and the occurrence of

CKD. This cohort will be assembled from employment and medical records; workers will be categorized according to several exposure definitions, and analyses will determine the incidence of abnormal test results, symptoms, disease, and death. We plan to use employment and payment records to construct a detailed employment history for individual workers. Due to the seasonal nature of the ISA's work, we anticipate linking annual work records for each worker (through their social security number) for each year worked and then compiling his or her complete work history. If necessary, annual screening records may be used to supplement data missing from employment records.

We plan to group workers into the following six occupational categories: 1) cane cutters; 2) seed cutters, planters, and weeders; 3) pesticide applicators; 4) factory workers; 5) office/administrative; and 6) other miscellaneous. Three main hypotheses regarding a possible occupational etiology for CKD will be examined: exposure to agrichemicals, volume depletion, and muscle damage. The latter two hypotheses are closely related and are unlikely to be distinguishable based on data abstracted from records, but information gleaned from work observation should provide a basis for determining the interplay between these two mechanisms. Therefore, for purposes of exposure categorization we have treated them as a single hypothesis. Job history data will be used to estimate exposure to agrichemicals. Job titles can be categorized according to potential for exposure to agrichemicals determined to have nephrotoxic potential based on toxicological review and information from company representatives, current and former workers, company records, our environmental sampling, and literature describing typical exposure patterns in sugar cane cultivation, including frequency and season of application. We will also use job titles to classify work intensity based on results from the work observation to examine volume depletion and muscle damage. In addition, because cane cutters are paid on a piecework basis, company payment records will be used to approximate the amount of tonnage cut, which will in turn be used to construct a measure of work intensity among cane cutters.

People who work at ISA are divided into three categories: permanent employees, temporary employees, and contractors. The size of the permanent workforce is about 600. Approximately 4000 workers—mainly cane cutters—are hired for the harvest months (November-May), and 800 temporary workers work the remaining six months.

Employment records are available since the 1960s. Annual screening for creatinine began in 1996. Therefore, this is the start year of follow-up for the cohort study with hypotheses involving creatinine level as the main outcome of interest. Follow-up will continue through December 31, 2010 (total follow-up time of 14 years).

We will review available medical records to obtain information on the occurrence of medical outcomes across the spectrum of kidney disease from abnormal kidney function test results to symptoms, disease incidence, and mortality. The three sources of medical records that we will use are:

- 1) ISA Hospital. Throughout the entire period of eligibility, the ISA has had a hospital on its grounds that has provided both inpatient and outpatient care free of charge for

current employees and their families and for retirees. Almost all medical records are on paper, and are stored on site.

2) Annual physical examinations for contracted employees (2003 to present). Prior to 2003, all persons working at ISA were employees of NSEL. Beginning in 2003, workers who harvested the cane were hired on an annual basis through subcontractors. The yearly physical exam results for this group have been obtained and stored separately. More recent records have been computerized, and plans are to computerize the earlier records as well, which may be completed in time for this study.

3) Local health centers and regional hospitals (2003 to present). Some workers who can no longer work because of high creatinine tests receive follow-up care at the local health center in Chichigalpa, which has a dedicated CKD unit, and also apply for government benefits available to them as a result of no longer being able to work. Regional hospitals (e.g. Hospital España in Chinandega) provide care for some workers as their disease worsens and are another source of medical information.

Access to these medical sources will allow us to compile a wide range of information that may serve as endpoints of interest (e.g., creatinine levels) or as important confounders that can be controlled (e.g., blood pressure). For example, the annual exams provide an ongoing measure of kidney function, assessed repeatedly for individuals. In addition, we can determine duration and course of CKD and its relationship to mortality.

Approximately 20,000 workers have been employed by ISA during the follow-up period covered by this study. Because most employment and medical records are not computerized, we do not believe that it is feasible to review the records of all workers employed during the follow-up period. An estimated 2,000-3,000 records of workers who have developed CKD appear to be available at ISA. A review of 4,000 randomly selected records would result in an expected 500-600 cases of CKD. The final sample size will be informed by the results of the feasibility/pilot study in consultation with a biostatistician. It will be necessary to engage a senior epidemiologist based in Nicaragua to help direct the project.

The study activities, which will require a minimum of 19 months, will be divided into two phases: a feasibility/pilot phase lasting five months and a main study phase lasting 14 months. We will first conduct a feasibility study that will include a detailed and critical review of existing occupational records, occupational exposure assessments and records, employment records, medical care facilities and available medical records. Understanding the availability and quality of these records will be an essential component of refining the proposed cohort study. During this phase we will also conduct a pilot study, which will be based on 50 occupational and 50 linked medical records, to determine the most efficient and feasible manner for conducting the main study. In particular, we will assess the organization of occupational and medical records and will pre-test record review and linkage procedures and data collection forms during this period. We will then prepare a preliminary report summarizing the availability, completeness, and quality of records and the feasibility of their use for a cohort study. The report will be reviewed by the Scientific Advisory Board.

Possible challenges: We have assumed that existing company, employment, environmental sampling, medical care facility and individual medical records are sufficiently detailed and valid to be able to conduct the above described study. While it is likely that basic information such as job title and dates of employment will be available, it is less clear whether more detailed data will be obtainable. For example, it is unclear if historical records on environmental sampling will be sufficient to construct a valid index of exposure to specific agrichemicals. Thus, depending on the results of the feasibility/pilot study, it is likely that proposed study design and protocol will be refined. Any important changes in scope will be submitted for review to the Scientific Advisory Board prior to implementation.

## 5. Medical Record Review

Review of medical records represents a potentially high yield source of data to help characterize the nature and medical correlates of kidney disease among former workers at the ISA. The primary sources of potentially available data were described above in the section on the Cohort Study of ISA Employees. Medical charts will also include data on screening for kidney disease. Serum creatinine measurement and urine dipstick is performed up to three times annually among ISA workers. For sugar cane cutters, testing occurs at the start of the season, in the middle of the season, and at the completion of the cutting season (three times in a 6 month period), although, per report of the physician staff at the ISA, the final test of the year often fails to occur for seasonal workers.

Chart review has the potential to help determine the cause of kidney disease, discriminating between risk factors for and presence of glomerular disease versus manifestations more consistent with tubulointerstitial disease. Specifically, we will be looking for evidence of tubulointerstitial disease due to medication use, glomerular disease, diabetes, hypertension, occupational-related exposures, and kidney stones. These chart reviews will be conducted as part of the data collection for the cohort study, and therefore will not require additional personnel or other costs.

## 6. Urinary protein determination in adolescents

The purpose of this activity is to determine the prevalence of CKD prior to subjects entering the work place as it is possible that CKD may be occurring in the general population but that sugar cane cutters have a more rapid progression to symptomatic CKD because of recurrent volume depletion and myoglobinuria. Accordingly, we are focusing on urine proteomics to identify early kidney disease. The work proposed consists of collecting urine samples from adolescents aged 12-16 years. Our hypothesis is that if there is epidemic chronic tubulointerstitial disease due to heavy metals, aristolochic acid, or other nephrotoxins, or from hereditary tubulointerstitial nephritis, early indicators of kidney damage, such as tubular proteinuria, will be manifest. With a prevalence of overt kidney damage of 5-15% as determined by low eGFR, one might expect more sensitive markers such as tubular proteinuria to be present in an even higher percentage. We will select subjects for this study to include

children of cane workers with known CKD, nieces and nephews of affected workers whose parents are not affected, and children whose parents have never worked in sugar cane. Approximately 100 children will be studied with equal numbers of males and females.

### 7. Post-mortem renal biopsy

There is potential utility in obtaining kidney biopsies early in the course of the disease or in people without clinical manifestations to determine if early pathologic abnormalities are present. However, since renal biopsy is associated with some risk, including death, and it is unlikely that a biopsy would alter the therapy for the renal disease, there are ethical concerns regarding performance of biopsies. One possible solution would be to obtain post-mortem renal biopsies in people dying from acute trauma, such as motor vehicle accidents. We understand that deaths from motorcycle accidents in people not wearing helmets are common and these victims are often young males, the group which is at risk for CKD.

We propose initially doing 10 post-mortem renal biopsies, processing the tissue only for light microscopy. Depending on the initial results we may want to obtain additional biopsies and include processing for immunofluorescence and electron microscopy. Additional evaluation could examine for aristolochic acid DNA adducts. It would take several months to identify and enlist the cooperation of appropriate hospital personnel and, perhaps, provide some education to the community. At least two groups have informed us of their plans to conduct biopsies on living persons with early kidney disease. Although we would like to discuss our ethical concerns with them, if they do choose to proceed with permission from ethics boards in Nicaragua, we would take advantage of the information they generated, and would likely not continue with postmortem biopsies.

Potential challenges: The logistical barriers, including cultural taboos, informed consent, recruiting hospital personnel to perform the biopsies, and tissue processing make this effort a formidable undertaking. Optimally we would require creatinine measurements on potential subjects to exclude significant kidney disease, and these measurements may not be available on accident victims. In the absence of knowing the specific cause of the epidemic CKD this effort is also largely exploratory. There are some potential toxins that can be specifically identified in the kidney, such as aristolochic acid, but for others the histologic findings may be non-specific. However, if done sufficiently early in the disease process, it could tell us whether we are dealing with a glomerular or tubulointerstitial disease. However, because the population on which we can perform biopsies may not be representative of the population at risk, any conclusions would be tentative. As discussed, access to the results of a planned broader kidney biopsy study would enhance our efforts.

## 8. Interviews

Certain exposures have the potential to cause CKD but are difficult to study in a manner that is likely to advance understanding of the likelihood that they are in fact a cause of CKD. For these exposures -- in particular, lija consumption, use of herbal medicines, occurrence of urinary tract infections (UTIs) and use of medications to treat UTIs and other common problems -- more information is needed about their constituents and patterns of use in the population before a realistic study plan can be developed. In order to obtain this information, we propose to interview persons who would have special knowledge in this area. Based on the information obtained, we will determine whether further activities regarding these two hypotheses are warranted and likely to bear fruit. If we come to a positive conclusion, we may propose additional activities. The types of persons we plan to interview for each hypothesis include:

- Lija: MINSA, law enforcement officials, physicians, and cooperative distributors and retailers
- Herbal medicines: botanists, toxicologists, cultural anthropologists, physicians, and local lay/traditional healers
- UTIs and medications: local physicians

In addition, interviewing persons with knowledge about work at the ISA (ISA personnel and current and former workers) will provide a better understanding of historical work patterns and exposures that can improve the environmental sampling and cohort study activities.

Therefore, we propose to conduct interviews for two purposes: 1) refining our data collection plans for what we consider to be immediate, high priority hypotheses and, 2) exploring hypotheses that we could not otherwise address.

Possible challenges: The limitation of interview data is that we rely on individuals as a source of information. However, this is also a strength of qualitative research in that the information provided by individuals is often not otherwise available. It is important to have well trained interviewers so that the information collected during interview sessions is as reliable as possible. To ensure this, our physician interviews will be piloted with physicians in Nicaragua.

## 9. Other Possible Activities

There are additional opportunities for study activities which our team has discussed or learned about in the process of developing our recommendations. Although we have not integrated them into our study plan as specific activities, we provide a list with a brief description so that readers of this report will have a more complete basis to provide input and suggestions:

1. Prospective cohort study among workers at ISA: Our proposed study is a retrospective cohort study, thus we are limited to information that has already been collected. However, beginning a prospective cohort study among current and new

employees would enable us to collect additional information and would also help determine to what extent the problem of CKD among workers at ISA is decreasing or increasing.

2. Collaboration with a second sugar company: Monte Rosa Sugar is located in the municipality of El Viejo in the department of Chinandega. Based on separate discussions with two company representatives, it appears that there is a perception that persons who work at Monte Rosa have an elevated rate of CKD. The representatives further indicated their support for this initiative to bring resources to studying the problem and expressed an interest in participating in some way. Conducting certain parallel activities as recommended in this report at a second company in the same region would widen the scope from a single company and help strengthen interpretation of the results.
3. Assessment of cumulative exposure to lead: A limitation of biological testing for lead levels using blood is that it only provides information on recent exposure. Cumulative lead exposure can be assessed in bone using x-ray fluorescence. The procedure is impractical to carry out on a large scale, but we may want to test a smaller group if there appears to be any evidence of significant lead exposure based on either environmental or biological testing.
4. Collaboration with a new prevalence and case-control study of CKD: The group at UNAN-Leon CIDS, in collaboration with the University of North Carolina is beginning to conduct a prevalence study with measurement of creatinine among 3,000 residents in the municipality of Leon. They will then use that population as the sampling frame for a case-control study of CKD, which will collect both biological samples and questionnaire-based information. Results from these studies, which appear to have been rigorously designed, can also provide data from a different population. Furthermore, providing funds to collect additional information beyond that currently planned (e.g., environmental sampling) could also increase the value of the study activities we undertake.
5. Initiation of prevalence studies in northeastern Nicaragua and Rivas: Tufts University School of Medicine, one of our sister schools in Boston, has an elective in which students provide medical care in Siuna, a town in northeastern Nicaragua where the primary economic activity used to be gold mining. Although this is the same activity as carried out in Larreynaga, the municipality in northwestern Nicaragua that along with Chichigalpa has the highest recorded rate of CKD mortality in the country, to our knowledge there is not a high rate of CKD in Siuna. It is possible that for very little additional cost, a cross-sectional prevalence study could be conducted there which would provide the first comparative data from outside the departments of Leon and Chinandega.

In addition, a physician affiliated with Boston University School of Medicine has close ties with the medical director of the regional hospital for Rivas, and they are both interested in conducting a prevalence study in that area. A modest amount of support could provide data from another area in the Zona del Occidente.

Most of these activities would require additional funding—some substantially more—and so may not be feasible. However, we hope that their inclusion here may spark

additional ideas and may even lead to ideas for alternative sources of additional funding.

## V. Timeline and Personnel

The timeline is intended to provide a general idea of when various activities would begin and be completed over the study period, which we estimate to be 2.5 years from January 2010, based on the assumptions that the recommended activities are approved by the parties soon after the issuance of this report and that there are no important obstacles to initiating work on the project. Although we estimate that it will take two years from initiation of study activities in February to completely finish the project, information obtained from individual activities will be available more quickly. One important potential obstacle is the time it will take to get Institutional Review Board approval from BU and from a Board in Nicaragua, likely MINSA. Occasionally, the process can take a long time, and we cannot conduct study activities that involve interaction with people or their confidential data until we receive approval. We will do our best to move the process along as quickly as possible.

Activity Description	Time period									
	2010					2011				
	Jan	Feb-Apr	May-Jul	Aug-Oct	Nov-Jan	Feb-Apr	May-Jul	Aug-Oct	Nov-Jan	
General preparation	X									
Environmental sampling		X	X	X	X					
Biological sampling			X	X	X					
Work observation		X	X	X						
Cohort study			X	X	X	X	X	X		
Medical chart review			X	X	X	X	X	X		
Urinalysis in adolescents				X	X	X				
Postmortem biopsies			X	X	X					
Key informant interviews		X	X							
Report preparation										X

We propose 12 people be involved as personnel in the project. Although a larger number than typical, it is necessary to accomplish the wide variety of activities proposed over a compressed timeframe. The 12 individuals fall into the following categories:

<b>Role</b>	<b># positions</b>
Principal Investigator (Epidemiology)	1
Epidemiology	2
Environmental Health and Community Training/Participation	2
Nephrology	2
Biostatistician	1
Project Director	1
Research Assistant (Boston)	1
Nicaraguan co-investigator	1
Research Assistant (Nicaragua)	1

In addition, note that two of the proposed personnel are from Nicaragua. The first is a research assistant who would carry out many of the logistical tasks associated with both study activities and managing visits by the study team. The second is a co-investigator for the project, most likely an epidemiologist, who can interact with the BUSPH team on a scientific basis and oversee activities in consultation with the BUSPH team. This position is critical to the success of the study as well as to efficiency (both fiscal and temporal). Although we will be making frequent trips and our Project Director will be spending a substantial amount of time in Nicaragua, there will inevitably be questions and issues that arise which can best be addressed by a Nicaraguan co-investigator. First, study activities will continue when nobody from the BUSPH team is present, and continuous scientific supervision is necessary. Second, it will be impractical and inefficient to require our presence to resolve every problem. Third, some problems will require knowledge of particular conditions and networks in Nicaragua, and a Nicaraguan co-investigator will have a better grasp of these conditions and thus be able to make more informed decisions.

## **VI. Conclusion**

The Dialogue process has created a unique opportunity to make great progress in the effort to determine the causes of the epidemic of CKD in Nicaragua and create the conditions for interventions aimed at preventing future cases. While many of the activities recommended in this report have been suggested by Nicaraguan investigators previously, they could not be implemented.

We appreciate the trust and cooperation shown to our team by all parties. It has allowed us to analyze the situation and propose an integrated set of activities addressing a range of hypotheses that we believe can move us a long way to the goal of stopping this epidemic. We have already benefited from the input of reviewers, and look forward to the same from the Dialogue partners and other involved and interested parties, to not only strengthen this report but all our activities on an ongoing basis.

The plan we have proposed is ambitious. We believe that, with the collaboration of the Dialogue partners and other involved and interested parties, we can accomplish much. However, we must also temper our expectations with the realism that CKD appears to a

complex, multifaceted problem in Nicaragua. We can not guarantee that our pursuits will find a single, explicit cause that will stop this epidemic: we can only hope that the information we uncover will lead directly to the development of actions to reduce the heavy burden of CKD and identify strategies to prevent future illness.

## I. INTRODUCTION

This report was produced as part of a contract that was issued to Boston University School of Public Health (BUSPH) by the Office of the Compliance Advisor/Ombudsman (CAO) of the International Finance Corporation (IFC) of the World Bank. The report is a component of a process that was initiated by a complaint filed by the Center for International Environmental Law on behalf of the Chichigalpa Association for Life (ASOCHIVIDA), an organization of individuals who formerly worked at the Ingenio San Antonio (ISA), which is owned by National Sugar Estates Limited (NSEL). The complaint alleged that the IFC failed to address the health and wellbeing of workers or the environment when delivering a substantial loan to NSEL. The loan was intended to expand NSEL's land and sugarcane processing capability, and the operational changes undertaken as a consequence of the loan were alleged to have resulted in harm to the workers and the environment, the primary example being an epidemic of chronic kidney disease (CKD), also referred to as chronic renal insufficiency (CRI).

By way of background, NSEL was founded in 1890 and has been continuously owned by the Pellas family except for a period during the 1980s when it was nationalized. The primary economic activity of NSEL has always been sugar cane cultivation, processing, and manufacturing; additional activities currently include ethanol, alcoholic beverage, shrimp and electric energy production. The operational center (Ingenio San Antonio, ISA) has always been located in the town of Chichigalpa in the department of Chinandega. The company currently owns approximately 24,000 hectares of land in Chinandega and Leon, and leases and operates an additional 15,000 hectares. In addition, it buys sugar cane from local independent growers to supplement its crops, which accounts for 30% of NSEL's total need. NSEL is the largest of four sugar companies in Nicaragua; it currently employs approximately 5,000 permanent and temporary workers and is the largest employer in Chinandega.

In response to the complaint filed by ASOCHIVIDA, CAO conducted a preliminary investigation, which resulted in the recommendation that a dialogue process (hereinafter referred to as "Dialogue") be initiated between representatives of ASOCHIVIDA and NSEL and facilitated by CAO. Dialogue meetings began in February 2009 and have continued on a regular basis. One of the early decisions taken by the participants was to focus activities in two areas:

1. Explore options for improved care for affected community members; and
2. Conduct a study to better understand the causes of CRI in the region.

As the first step in addressing the second objective, a Terms of Reference (TOR) was prepared and released for a Scoping Study to (1) assess whether further study was necessary, feasible, and likely to be successful in answering the following two questions:

- 1.) What are the causes of CRI in the Western Zone (Zona del Occidente) of Nicaragua – an area that includes the Ingenio San Antonio and its sugarcane plantations?
- 2.) Is there any relationship between the practices of the Ingenio San Antonio and the causes of CRI?

and (2) to recommend activities that would lead to fulfillment of this objective.

The TOR further specified that the study team should:

1) Review the existing information available on CKD in Nicaragua in order to: (i) assess whether the available information was sufficient to answer either of the two questions; (ii) if not sufficient, identify gaps and limitations in the available information that prevented making a causal inference; (iii) determine whether additional study could reasonably lead to causal inference; and (iv) guide identification of study design options that could obtain the necessary information.

2) Make a fact-finding trip to Nicaragua in order to: (i) meet with ASOCHIVIDA and NSEL dialogue table participants to refine questions to be addressed; (ii) meet with other key stakeholders (MINSAs, medical providers, researchers) to better understand prior work, current situation, and potential resources for study activities; (iii) conduct a preliminary assessment of local laboratory and hospital capacity to collect and analyze samples; and (iv) identify potential partners for collaborative efforts during follow-up study implementation

3) Synthesize collected information and prepare a presentation of study design options and recommendations that is based on the information generated in Tasks 1 and 2 and best professional judgment

4) Present and discuss the study design options and recommendations at a workshop with the participants.

5) Based on the results of the previous four tasks, prepare a study design options report which presents proposed study activities that will contribute to answering the two causal questions posed by the dialogue table participants.

BUSPH was selected in April by the Dialogue participants to conduct the Scoping Study. In order to accomplish these activities, we assembled a team consisting of the individuals listed in Table 1.

During the three months since BUSPH began involvement in June 2009, we have reviewed all available information that we were able to locate on CRI in Nicaragua, identified numerous hypotheses, conducted two week-long visits in June and July to Nicaragua to meet with all stakeholders, and developed recommendations. This Scoping Study Report provides a summary of all of these activities.

**Table 1: BUSPH Scoping Study Team Members**

Name/title	Affiliation	Relevant background/ expertise
Daniel Brooks, DSc	BUSPH	Epidemiology, prior studies on CKD in Nicaragua
Ann Aschengrau, PhD	BUSPH	Epidemiology, environmental epidemiology studies
Michael McClean, ScD	BUSPH	Occupational and environmental exposure assessment
Madeleine Scammell, DSc	BUSPH	Environmental health, community participation in research
James Kaufman, MD	BU Medical School	Nephrologist, clinical outcomes research
Daniel Weiner, MD, MS	Tufts Medical Center	Nephrologist, clinical outcomes research
Lesley Stevens, MD, MS	Tufts Medical Center	Nephrologist, clinical outcomes research
Peter Soderland, MD	BU Medical School	Nephrology Fellow
Oriana Ramirez, MD, MPH	Universidad Autonoma Madrid	Preventive medicine, prior studies on CKD in Nicaragua
Bruce Cohen, PhD	Brookline-Quezalguaque Sister City Committee	Epidemiology, prior studies on CKD in Nicaragua
Peter Stringham, MD	Brookline-Quezalguaque Sister City Committee	Prior studies on CKD in Nicaragua
Valerie Bouchet, DSc	BU Medical School	Environmental Health
Casey Rebholz, MPH	Tulane University	Medical student and PhD candidate in epidemiology, prior studies on CKD in Nicaragua
Matthew Tobey,	BU Medical School	Medical student, prior studies on CKD in Nicaragua
Katie Biello, MPH	Yale University	PhD candidate in epidemiology, prior studies on CKD in Nicaragua
Rachel Pitek, MPH	BUSPH	Spanish language, technical and logistical assistance

Brief summaries of most of the relevant documents are provided in the Appendix. These summaries were prepared by research assistants for our internal use and have not been modified. Therefore, they should not necessarily be relied on as a substitute for a review of the document.

During our trips to Nicaragua, we met with representatives from the following institutions and organizations:

- NSEL
- ASOCHIVIDA
- MINSA
- Hospital Espana
- Chichigalpa Health Center
- UNAN-Leon CISTA
- UNAN-Leon CIDS
- Coen Foundation

In addition, we met with Drs. Luis Callejas Callejas (now a member of the National Assembly) and Carlos Alonso Medrano (now a physician for the Monte Rosa sugar company), who conducted some of the early studies on CKD in the northwestern region of Nicaragua.

The context in which this Scoping Study has been carried out is important to understanding the nature of this report. There are two key ways in which this project is unique compared to a more typical research scenario in which we would typically propose a large scale study that is conducted over many years.

First, this project grows out of a specific conflict and an immediate need for answers. Whatever the cause, there is an excess of people dying from CRI in the northwestern region of Nicaragua. Accordingly, there are very real and potentially profound public health implications associated with this project, such that our approach could be perhaps be characterized more as a response to a public health emergency rather than as a research proposal.

Second, this project is being conducted as part of a carefully navigated process in which multiple stakeholders are engaged. As such, an important component of this effort is to ensure that we address the issues that are most relevant to the participants in this dialogue process. It is also important that participants are involved in each stage of the investigation, from agreeing to the proposed study design to having input into the field investigations and observing various study activities to receiving regular updates on progress, so that they are ultimately comfortable with accepting the results.

Accordingly, given the uniqueness of this project, we have not proposed a comprehensive large-scale study that would be conducted over a five year period. We have instead proposed shorter term discrete steps that have been designed to address key data gaps, the concerns of the dialogue participants, and in our view, have the potential to yield high impact information.

Finally, as a further consequence of the importance of making rapid progress toward answering the questions posed by the Dialogue, we have operated within a very tight timeframe in the collection and review of information, development of recommendations, and preparation of this report. Therefore, our goal has necessarily been to focus on the available literature and other information only to the extent they will help improve our understanding in order to develop proposed activities. We also recognize that the activities themselves are not as fully detailed as they will eventually need to be. We view this report not as the final word but as an important step toward a plan to identify the causes of this devastating disease, and we look forward to input from stakeholders, reviewers, and other interested parties, which will undoubtedly result in an even stronger plan.

## II. CKD IN NICARAGUA: STATE OF KNOWLEDGE

### A. CKD as global health problem

Reflecting its rising incidence and prevalence, chronic kidney disease (CKD) is a major international public health concern. Recent worldwide initiatives have attempted to garner attention for CKD by emphasizing that the condition is “common, harmful, and treatable” (Levey AS, 2007). In the United States, as many as 26 million adults may have CKD, an increase from approximately 10% of the US adult population between 1988 and 1994 to over 13% just one decade later (Coresh J, 2003; Coresh J, 2007). Similar rates are seen worldwide, with CKD prevalence of 13% in Beijing, China (Zhang L, 2008) and 16% in Australia (Chadban SJ, 2003). In the United States, the dramatic rise in the prevalence of CKD likely reflects similar increases in obesity rates and sequelae of obesity—namely, diabetes, hypertension, and cardiovascular disease (Coresh J, 2007). The prevalence of CKD, as well as its associated costs, is expected to continue to increase (Levey AS, 2009).

CKD is defined by either a reduced glomerular filtration rate (GFR  $<60$  mL/min per  $1.73\text{m}^2$ ) or by evidence of kidney damage. Early stages of CKD manifest with only kidney damage in the setting of overtly intact GFR; the most common marker of kidney damage is albumin in the urine while a second common marker is hematuria. Stage 3 CKD is defined by GFR between 30 and 59 mL/min per  $1.73\text{m}^2$  and is the stage at which clinical sequelae of CKD are often first appreciated. Stage 4 CKD is defined by GFR of 15 to 29 mL/min per  $1.73\text{m}^2$ , and stage 5 by GFR  $<15$  mL/min per  $1.73\text{m}^2$  or requirement for kidney replacement therapy (Table 2). In developed nations, a minority of individuals with CKD develop end stage renal disease (ESRD) requiring kidney replacement therapy, with the majority often dying prematurely of cardiovascular disease.

There is increasing worldwide awareness of CKD as a significant public health problem. In the past, the focus in developed countries has been primarily on ESRD with its attendant health and financial burdens. In the United States in 2006, approximately 355,000 patients with ESRD were receiving dialysis therapies with an additional 150,000 individuals with functioning kidney transplants (Figure 1) (USRDS 2008 Annual Data Report). Medicare, the federal insurance program in the US which covers people age 65 and older or those with ESRD, bears the brunt of this cost, with dialysis related expenditures of over \$20 billion with private insurers contributing another \$11 billion. However, earlier stages of CKD disease also have significant health consequences, including hypertension, anemia, and accelerated cardiovascular disease. There has been increasing emphasis on the recognition of early CKD and the institution of interventions to slow its progression.

**Table 2. Stages, Description, Prevalence and Clinical Action Plan for CKD and Individuals at Increased Risk of CKD**

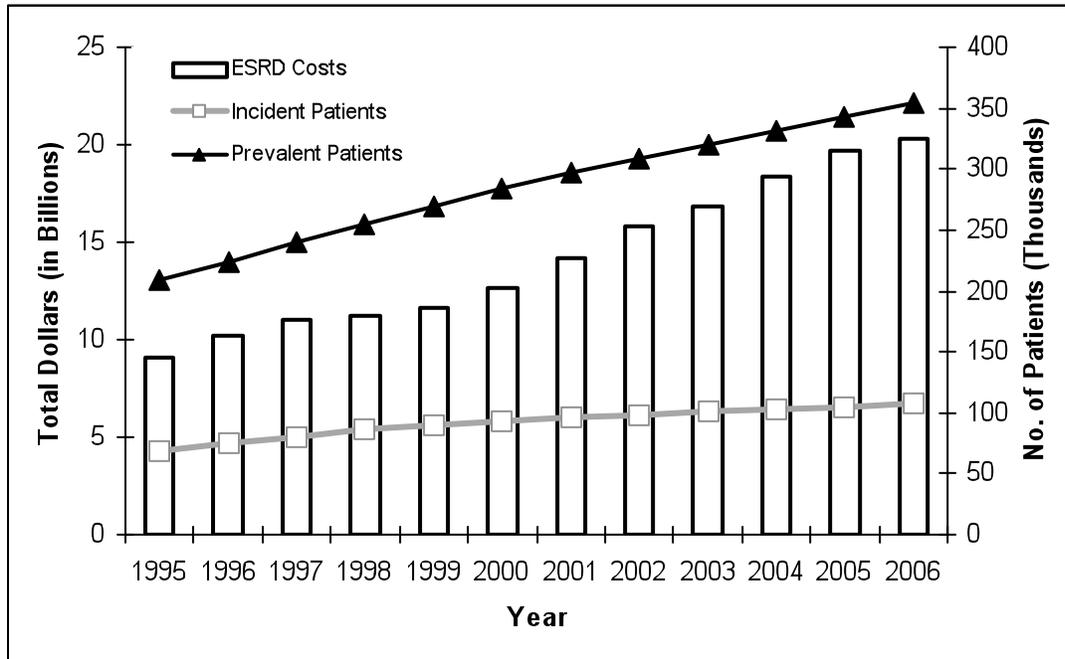
Stage	Description	GFR ( <i>mL/min per 1.73 m<sup>2</sup></i> )	US Prevalence N [1,000s]	Clinical Action Plan
---	Increased risk for CKD <ul style="list-style-type: none"> <li>• Age ≥60 years</li> <li>• Hypertension</li> <li>• Diabetes</li> <li>• Cardiovascular disease</li> <li>• Family history of CKD</li> </ul>	Not applicable	Risk factor prevalence <ul style="list-style-type: none"> <li>• Age &gt;60: 50,600 (23.2%)</li> <li>• Hypertension: 65,000 (32.3%)</li> <li>• Diabetes: 20,600 (9.6%)</li> <li>• CVD: 71,300 (34.2%)</li> <li>• Family History: unknown</li> </ul>	Screening Primary prevention and CKD risk reduction, including blood pressure and glycemic control
1	Kidney damage with normal or increased GFR	≥90	3,600 (1.8%)	Diagnosis of CKD cause Education about CKD Treatment of comorbid conditions Evaluation of risk for and assessment of rate of progression Treatment to slow progression
2	Kidney damage with mild decrease in GFR	60-89	6,500 (3.2%)	CVD risk reduction Referral to nephrologist for rapid kidney disease progression
3	Moderately decreased GFR	30-59	15,500 (7.7%)	Evaluate and treat complications, including bone and mineral disorder, anemia, and dyslipidemia Consider discussion of kidney replacement therapy options, particularly in late stage 3 or rapid progressors
4	Severely decreased GFR	15-29	700 (0.4%)	Prepare for kidney replacement therapy Place vascular access or develop plan for peritoneal access or pre-emptive transplant Referral to nephrologist
5	Kidney failure	<15 or dialysis	~400 (0.2%)	Kidney replacement therapy

The clinical action plans are additive such that the plans documented in earlier stages still apply in later stages of CKD. To classify an individual with CKD, reduced GFR or kidney damage should be present for at least 3 months. Original adapted from (3, 6, 11); reproduced from (12).

The causes of CKD are numerous (Table 3) and typically are divided into vascular, glomerular, tubulointerstitial and obstructive etiologies. This classification not only provides a convenient pathophysiologic framework, but also may relate to clinical manifestations. Vascular and obstructive causes are relatively limited in number and more readily diagnosed, while glomerular and tubulointerstitial diseases have a far broader spectrum of causes. Glomerular diseases often present with typical urine findings, including proteinuria as well as the presence of red blood cells or red blood cell casts in the urine sediment. Proteinuria, when quantified, is often greater than 2 gm/day and consists primarily of albumin. Tubulointerstitial diseases may also have proteinuria; however, this urinary protein is often not albumin, but rather includes proteins of tubular cell origin and the total amount excreted is usually much lower than that seen in glomerular diseases. White blood cells, renal tubular cells, and white blood cell casts may be present in the urine sediment in tubulointerstitial diseases, but often the sediment is unrevealing. Clinical features distinguishing the different etiologies are more likely to be present early in the course of CKD. As CKD progresses to advanced

stages, the specific etiology is often not apparent clinically, and even biopsies may be of limited yield because late stages of most kidney diseases, regardless of etiology, manifest with extensive scarring and fibrosis.

**Figure 1. Incidence and prevalence of dialysis in the United States and its associated costs**



The major causes of kidney disease and subsequent kidney failure in the US are diabetes (accounting for 44.4% of incident cases of kidney failure in 2006) and hypertension (accounting for 26.8%) (Collins AJ, 2009), both of which are increasingly common in an increasingly overweight US population (MMWR Morb Mortal Wkly Rep. 2008). Conditions accounting for the remaining 29% include primary glomerulopathies like focal glomerulosclerosis and IgA nephropathy, inherited conditions like polycystic kidney disease, and autoimmune conditions like lupus. These diseases have also been identified as important causes in developing countries.

Because of limited data, less is known about the causes of CKD in developing countries. There have been several screening studies of randomly selected community based populations in the Democratic Republic of Congo, India, southern China, Vietnam, and Jalisco, Mexico which have found a prevalence of proteinuria of 2-11% and a prevalence of an eGFR <60 ml/min/1.73 m<sup>2</sup> of 3-16%, not dissimilar to rates reported from the United States and western Europe (Sumaili, 2009; Singh, 2009; Chen, 2009; Ito, 2008; Gutierrez-Padilla, 2009). While these studies do not attempt to establish a diagnostic etiology, they generally note a high prevalence of hypertension and diabetes in the affected population. While it has been observed that diabetes is a less common cause of CKD in developing countries, perhaps because of a lower prevalence of obesity, it still accounted for 28% of the cases of CKD among 14,796 patients studied in an outpatient nephrology unit in India (Agarwal, 2000). The most common cause in this study was chronic glomerulonephritis, seen in 49% of this

referral population. The spectrum of disease in this population likely is subject to referral bias, since patients with hypertension may less likely be referred.

**Table 3. Differential approach to CKD**

Classification	Condition	Associated Disease States	Manifestations	
V A S C U L A R	Main Renal Arteries	Renal artery stenosis Fibromuscular dysplasia	Often accompanied by hypertension	
	Intrarenal vasculature	Thrombotic microangiopathies Benign and malignant nephrosclerosis Atheroembolic disease	Minimal albuminuria, often with bland urine sediment	
	Renal vein	Renal vein thrombosis	May be a cause of or be caused by nephrotic syndrome	
G L O M E R U L A R	Focal GN	Age <15 yo	Post-infectious, IgA, TMBD, Hereditary nephritis, HSP, mesangial proliferative GN	Active sediment without reduced GFR or nephrotic syndrome
		Age 15-40 yo	IgA, TBMD, SLE, Hereditary nephritis, mesangial proliferative GN	
		Age >40 yo	IgA	
	Diffuse GN	Age <15 yo	Post-infectious, MPGN	Active sediment with reduced GFR and variable proteinuria
		Age 15-40 yo	Post-infectious, SLE, RPGNs, Fibrillary GN, MPGN	
		Age >40 yo	RPGNs, vasculitides, fibrillary GN, Post-infectious	
	Nephrotic Syndromes	Age <15 yo	MCD, FGS, mesangial proliferative GN	Heavy proteinuria, usually bland sediment
		Age 15-40 yo	FGS, MCD, Membranous, Diabetes, Pre-eclampsia, Late stage post-infectious	
		Age >40 yo	FGS, Membranous, diabetes, MCD, IgA, Amyloid/LCDD, HTN/nephrosclerosis, Late stage post-infectious	

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Classification	Condition	Associated Disease States	Manifestations
TUBULOINTERSTITIAL	Drugs and toxins	Analgesic nephropathy (non-steroidal anti-inflammatory drugs)	Minimal albuminuria, usually bland sediment, even in presence of reduced GFR; tubular proteinuria?
		Aminoglycoside associated nephrotoxicity	
		Aristolochic Acid	
		Calcineurin inhibitors	
		Chemotherapeutic agents (Cisplatin, nitrosureas)	
		Contrast induced nephropathy	
		Lithium	
		Metals (Lead, cadmium, arsenic, uranium, ?mercury)	
		Ochratoxin	
		Pigment nephropathy (rhabdomyolysis)	
Hereditary		Fabry Disease	Tubular or glomerular manifestations; rare
		Hereditary nephritis (Alport syndrome)	Often accompanied by hearing impairment; male predominance
		Medullary Cystic Disease	Indolent; difficult to diagnose
		Polycystic Kidney Disease	Massively enlarged cystic kidneys
Autoimmune		Sarcoid	Highly variable, usually with other systemic manifestations and typically accompanied by marked hypertension
		Scleroderma	
		Sjogren's syndrome	
		SLE	
Metabolic		Other vasculitides	Minimal albuminuria; usually bland sediment, even in presence of reduced GFR; tubular proteinuria?
		Cystinosis	
		Hyperuricemia	
		Hyperoxaluria	
		Hypokalemia	
Other		Nephrocalcinosis	Minimal albuminuria, usually bland sediment, even in presence of reduced GFR, tubular proteinuria?
		Chronic infections, ureteral reflux	
		Chronic ischemia, acute tubular necrosis	
		Hypertension	
		Lymphoproliferative diseases and myeloma	
Ureteral		Sickle cell disease	May have hematuria, but otherwise bland sediment
		Bilateral obstructing stones (or unilateral if single functional kidney)	
Bladder		Malignancy	
		Strictures	
		Retroperitoneal fibrosis	
		Benign or malignant prostate enlargement	
		Urethral stricture	
Bladder		Neurogenic bladder	
		Infections	
		Medications	

**Table 3 legend.** FGS, focal glomerulosclerosis; TMBD, thin basement membrane disease; HSP, Henoch Schonlein purpura; GN, glomerulonephritis; SLE, Systemic lupus erythematosus; MPGN, membranoproliferative glomerulonephritis; RPGN, rapidly progressive glomerulonephritis; MCD, minimal change disease; LCDD, light chain deposition disease; HTN, hypertension

Geographic variations in the prevalence of CKD have been noted. Some of these, such as the increased prevalence in Pima Indians and Australian aborigines, have been linked to a high prevalence of diabetes and possibly low birth weight (and attendant decreased renal mass) in these populations (Hoy, 2000). In other populations, environmental toxins, particularly heavy metals, have been identified as a cause of CKD. Although heavy metal induced chronic kidney disease usually occurs through occupational exposure in industrial workers, it has also been identified in populations with contaminated food or water supplies. Examples include the occurrence of cadmium nephropathy in the Jinzu River basin in Japan, associated with the contamination of rice, and nephropathy from organic mercury poisoning along Minamata bay in Japan, associated with the ingestion of mercury contaminated fish (Jarup, 2002; Iesato, 1977).

Other environmental toxins have been identified as causes of CKD. In Tunisia, contamination of the food supply with ochratoxin A, a nephrotoxic mycotoxin, has been linked to an increased prevalence of CKD (Abid, 2003). One of the most fascinating examples of an environmental toxin as a cause of chronic kidney disease has been the identification of an association between aristolochic acid with both Balkan endemic nephropathy (BEN) (Bamais, 2008; Debelle, 2008) and Chinese herbal nephropathy. Although BEN was first described in the 1950's, its cause remained unknown for many years. A form of chronic interstitial nephritis, it only affects individuals residing in certain endemic regions along the Danube River in the Balkan countries of Bosnia, Bulgaria, Croatia, Romania and Serbia. One of the striking characteristics of the disease is the clustering in villages, families and households. All affected regions consist of villages built on the alluvial plains of the Danube River, although affected villages may be as close as 2 km from unaffected ones. Another feature of the disease is its long incubation period. Affected individuals must live in the area for 15-20 years. Therefore, it has neither been identified in children nor in adults who leave the area before reaching the age of 20 years. Finally, affected individuals are at increased risk for developing uroepithelial tumors.

In 1993, an outbreak of kidney failure was identified among Belgian women who received a Chinese herbal medicine for weight loss at a clinic in Brussels. The toxic product was identified as aristolochic acid, produced from the seeds of *Aristolochia clematitis* (birthwort). The clinical manifestations of the disease in these women were remarkably similar to those seen in Balkan endemic nephropathy, with similar morphologic characteristics and the occurrence of uroepithelial tumors. In 1967, Kazantis had proposed that Balkan nephropathy was caused by the contamination of the baking flour in endemic areas by seeds of *Aristolochia clematitis*, but this hypothesis had not garnered much attention. However, after the identification of Chinese herbal nephropathy, increasing interest in this toxin as a cause of Balkan nephropathy ensued, with the subsequent identification of aristolochic-specific DNA adducts in urinary tract tissue from patients with Balkan nephropathy and the development of an animal model. Therefore, it seems likely that contamination of flour with aristolochic acid is a likely cause of Balkan nephropathy.

Finally, there are recent reports of a high prevalence of CKD in parts of Sri Lanka with an increased prevalence in young male, agricultural workers. Described risk factors for CKD in Sri Lanka include being a farmer, using pesticides, drinking well water, a family history of renal dysfunction, use of ayurvedic treatment, and a history of snakebite (Wanigasuriya, 2007). A recent report suggests a potential relationship to elevated cadmium levels due to cadmium-contaminated agrochemicals in rice and freshwater fish (Bandara, 2008). This data overall remains extremely speculative and of limited quality.

One major factor with all of these ‘outbreaks’ of CKD is that, despite similar exposures to toxins like aristolochic acid or even chronic diseases like diabetes and hypertension, not everyone with exposures develops CKD. This pattern indicates that there are likely multiple factors involved in each stage of kidney disease (Levey, 2007). These can broadly be conceptualized as development factors and progression factors (Table 4). Development factors include **susceptibility factors** (which increase vulnerability to kidney damage, with examples including older age, family history/genetic predisposition, and even elements like volume depletion) and **initiation factors** (which actually cause kidney damage, with examples including diabetes, autoimmune diseases and toxins) **Progression factors** cause worsening damage and progressive decline in GFR, with examples including higher blood pressure, smoking and poor diabetes control. Certain factors, with sustained exposure, may even fall into more than one of these categories.

**Table 4. Risk factors for CKD**

<b>Factor Category</b>	<b>Definition</b>	<b>Examples</b>
<i>Development</i>		
Susceptibility	Increase susceptibility to kidney damage	Older age, family history of CKD, low kidney mass/low birth weight, minority status, low SES, volume depletion
Initiation	Directly cause kidney damage	Diabetes, hypertension, autoimmune diseases, systemic infections, recurrent urinary tract infections, nephrolithiasis, obstruction, drug toxicity
<i>Progression</i>	Cause worsening kidney damage and more rapid decline in GFR	Higher levels of proteinuria, higher blood pressure, poor diabetes control, smoking, cardiovascular disease

## **1. Characteristics of renal disease in Nicaragua**

Published peer-reviewed data on the characteristics of CKD in Nicaragua are unavailable; however, a number of unpublished studies were available to the study team. These data were in the form of meeting presentations, reports, submitted articles, and abstracts, and provide a reasonably consistent picture of the problem. The characteristics are summarized here. In Chinandega, 90% of described cases occurred in men, the majority of whom were 21-45 years old (Marín Ruiz, 2006). Only 3% had diabetes and 4% had arterial hypertension. The male predominance, young age at

onset, and the low prevalence of the common causes of CKD in developed countries (diabetes and hypertension) in affected patients in Nicaragua are supported by similar studies from other sugar cane growing areas (Torres, 2008; MINSA, 2002), although some of these studies do report hypertension in up to two-thirds of patients. Notably, hypertension is both a cause and a common complication of CKD and it is unclear from these reports when in the course of the disease the presence of hypertension was assessed.

As discussed, the degree of proteinuria is an important clue in the differentiation of glomerular from tubulointerstitial diseases, with the former generally having high-grade proteinuria. There are limited data on the presence of proteinuria in affected patients, but in discussions with physicians in Nicaragua, including several nephrologists, dipstick proteinuria was minimal (typically 1+ or below) and white cells were not infrequently present in the urine, features consistent with tubulointerstitial disease (personal communication, Dr. Erwin Reyes, Chichigalpa Health Center).

There is a single biopsy study (Zelaya, 2000) reporting results of 15 kidney biopsies. Of these, 14 revealed extensive fibrosis and the 15<sup>th</sup> showed chronic glomerulonephritis with granular and linear IgG and IgM deposits. This study highlights the difficulty of establishing a specific diagnosis in patients with advanced disease. Similarly the results of ultrasound studies have not provided specific diagnostic information, generally showing small kidneys without evidence of structural abnormalities, although some observers report a high prevalence of kidney stones.

Activities we propose as part of this study—review of medical records and conduct of postmortem biopsies on younger males without advanced CKD—should provide more information on the characteristics of CKD in Nicaragua.

## **B. Data on CKD in Nicaragua**

Both vital statistics data and reports from studies are available from Nicaragua and other Central American countries, a number of which have been reviewed in a report prepared for a 2005 workshop on CKD in Central America (Cuadra, 2006). The great majority of studies were conducted in Nicaragua, but elevated rates of CKD, with a similar demographic profile as seen in Nicaragua, have been reported in other Central American countries, particularly in low-altitude regions in the Pacific Region ranging from southern Mexico to Honduras, which suggests that the problem may be regional in scope (Dominguez, 2003; Cerdas, 2005; Cuadra, 2006; Garcia-Trabanino, 2005).

In line with the workplan requested by CAO, we have not prepared an exhaustive review of the literature but rather focused on determining what is known about CKD, strengths and limitations of the studies done to date, and gaps in the knowledge base that can be addressed by additional study activities.

Information on CKD in Nicaragua primarily comes from government data on mortality and cross-sectional prevalence and (retrospective) case-control studies. (There are also a number of case report series, but we have not included them in this review.) To our

knowledge, with the exception of two intervention studies conducted among ISA workers (Acosta, 1997; Zepeda, 2007), there are no intervention, cohort, or incident case-control studies.

In this review, we focus on two basic sets of questions: (1) what are the basic epidemiologic characteristics of CKD in Nicaragua, and (2) what are potential causes of excess CKD in Nicaragua. Information on the first question derives from government mortality statistics and cross-sectional studies that measured prevalence of CKD (we are not aware of any studies that have measured CKD incidence). Information on the second question derives from cross-sectional and case-control analyses.

## **1. Mortality Statistics**

There are two main strengths of mortality data. First, as part of the vital records system, it is ascertained on an ongoing basis and so its availability is not dependent on special studies. Second, it is the only source of national data available on CKD in Nicaragua. There are also important and well-known limitations of mortality data, which are discussed later.

Table 5, and figures 2 and 3 show CKD-specific mortality rates at the regional (department) level for 1992 – 2005 both overall and by sex. These data, gathered from the 2005 MINSa report and indicate the following:

1. Mortality due to CKD appears much higher in Leon and Chinandega than other departments and the country as a whole throughout the time period. At the same time, mortality rates due to CKD in most of the other departments in the Pacific region were also somewhat elevated in 2002 and 2005. In 2005, the overall rates (per 100,000 inhabitants) in Leon and Chinandega were 50 and 41, respectively, compared to 11 in the country as a whole. The increased rate in Leon and Chinandega was seen in all age groups. Chinandega has the highest death rates of any department due to CKD among ages 15-49, while Leon was highest among age  $\geq 50$ .
2. The mortality rate due to CKD (per 100,000 inhabitants) has increased over time in the country from approximately 4.5 in 1992 to 11 in 2005. The greatest increases in absolute terms by far have occurred in Leon and Chinandega (Granada has also shown a substantial increase). The increases in these departments likely accounts for a substantial proportion of the overall increase in the national rate.
3. The mortality rate among men is much higher than among women. In 2002, the male:female ratio across the whole country was approximately 4:1. In Leon and Chinandega, the male:female ratio was approximately 6:1; correspondingly, in many other departments, there is little or no apparent male excess. Departments with a male excess tend to be in the Pacific region. Although mortality rates among women were much lower than men, in 2002 rates for women were still

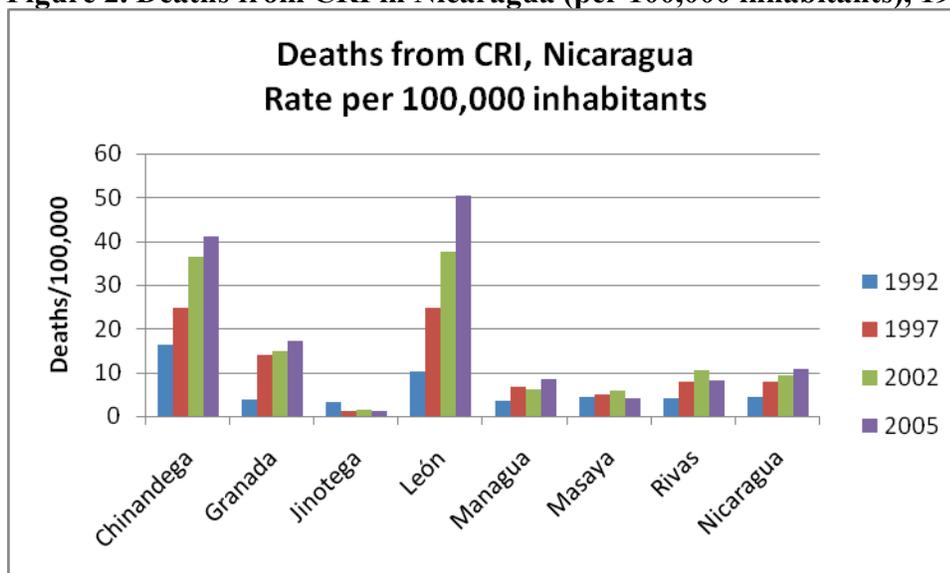
highest in Leon and Chinandega, and were also elevated in Granada and Rivas, all departments in the Pacific region.

**Table 5. Deaths from CRI in Nicaragua by department (rate per 100,000 inhabitants)**

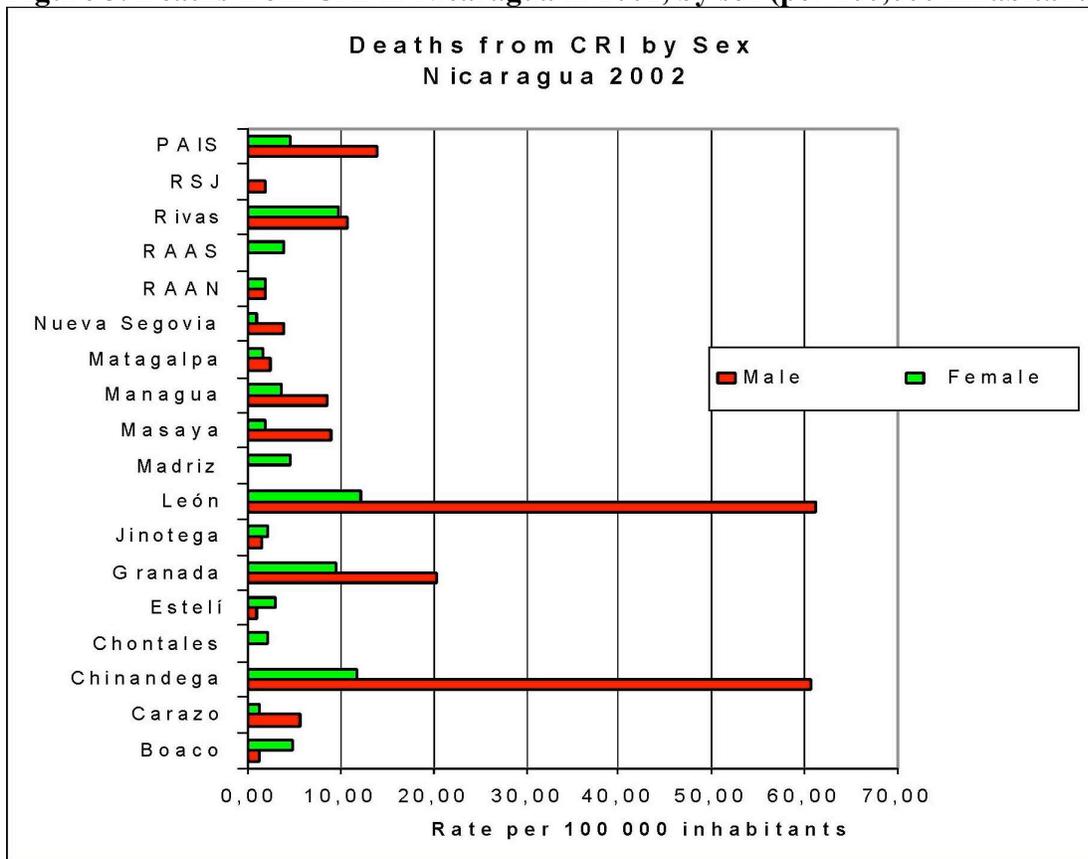
	1992*	1997*	2002*	2005
Boaco	3.8	4.8	3.0	4.0
Carazo	3.7	2.7	3.5	7.8
Chinandega	16.3	24.7	36.3	41.2
Chontales	1.4	1.6	1.1	3.9
Estelí	3.4	2.3	2.0	3.5
Granada	3.7	14.1	14.9	17.2
Jinotega	3.2	1.3	1.5	1.2
León	10.2	24.9	37.5	50.3
Madríz	2.2	6.6	2.2	0.8
Managua	3.4	6.6	6	8.5
Masaya	4.5	4.9	5.7	4.1
Matagalpa	0.8	2.2	2.2	3.6
Nueve Segovia	1.5	0.6	2.4	1.0
RAAN	0.8	0.6	2.0	1.0
RAAS	0.0	12.2	2.0	1.3
Río San Juan	0.0	0.0	1.0	1.0
Rivas	4.1	8	10.4	8.3
<b>Nicaragua</b>	<b>4.5</b>	<b>7.9</b>	<b>9.3</b>	<b>10.9</b>

\* Data for these years was estimated from graphs

**Figure 2. Deaths from CRI in Nicaragua (per 100,000 inhabitants), 1992-2005**



**Figure 3. Deaths from CRI in Nicaragua in 2002, by sex (per 100,000 inhabitants)**



## 2. Prevalence of CKD

CKD is defined by the presence of kidney damage or decreased kidney function as described in Section I.A. Therefore, ascertainment of the prevalence of CKD requires assessment of both parameters.

Three studies conducted in Nicaragua and other Central American countries used proteinuria measured by dipstick as a measure of kidney damage (Alonso, 2003; Dominguez, 2003; Garcia Trabanino, 2005). However, these studies have several limitations. In developed countries such as the U.S., diabetes and hypertension are the most common causes of CKD. As such, the most common marker of kidney damage, particularly early in its course, is albuminuria or total proteinuria. However, as diabetes and hypertension are unlikely to be the primary causes of CKD in Nicaragua, measures of glomerular proteinuria may be less informative. Additionally, the specificity of proteinuria ascertained by a urine dipstick is less certain than with quantification of albuminuria (or total proteinuria) after accounting for urine concentration.

Measurement of serum creatinine concentration allows estimation of glomerular filtration rate (GFR), the primary measure of kidney function. Critically, serum creatinine does not rise until late in CKD, reflecting the presence of substantial functional reserve in the kidneys. We were able to identify 10 studies that measured prevalence using serum

creatinine measurement (Callejas Callejas, 2003a; Callejas Callejas 2003b; Callejas Callejas 2003c; Siqueira, 2003; Lopez Arteaga, 2005; Torres, 2007; Torres, 2008a; Torres, 2008b; Aragon, 2009; Brookline Sister City Committee, 2008). The most recent studies (Torres, 2007; Torres, 2008a; Torres, 2008b; Aragon, 2009; Brookline, 2008) have used estimated GFR (eGFR) to define stages of CKD (see Table 6) The eGFR is typically determined by a formula that includes serum creatinine, sex, age, and race (dichotomized as Black versus non-Black) (Levey, 2001), and is a standard measure used in epidemiological studies. Although it is a more accurate measure of kidney function than serum creatinine alone as it accounts for non-kidney factors that affect the serum creatinine level, 80% of the GFR estimate is dependant on the creatinine component of the formula; accordingly, studies using serum creatinine or eGFR should provide similar comparative information particularly if they focus on a single demographic. However, it is difficult to make a direct comparison of absolute prevalence rates using the two different methods, especially if different studies use different serum creatinine rates to define a case.

Only one of these 10 studies was conducted outside the departments of Leon and Chinandega, in the north-central department of Jinotega (Siqueira, 2003). Of note, none have yet appeared in the peer-reviewed literature. All studies conducted prior to 2007 were based on convenience samples and/or in special populations (e.g., agricultural workers), and have been summarized elsewhere (Cuadras, 2006). Four studies used the same case definition of a serum creatinine of  $\geq 1.5$  mg/dl (Callejas Callejas, 2003a; Callejas Callejas 2003b; Callejas Callejas 2003c; Siqueira, 2003). The population assessed in these studies, along with the sample size, sex distribution, and prevalence are summarized in Table 6:

**Table 6. Studies using serum creatinine of 1.5 mg/dl as CKD case definition**

Study	Population	Sample Size	Male %	CKD %
Callejas Callejas, 2003a	Workers at Monte Rosa sugarcane "ingenio" (El Viejo, Chinandega)	2000	n/a	10%
Callejas Callejas, 2003b	Workers living in non-sugarcane areas and no history of working in sugarcane (Chinandega)	326	100%	7%
Callejas Callejas, 2003c	History of work and current residence in one of 6 communities in Chinandega or 3 in Leon	997	85%	10%
Siqueira, 2003	Workers at 15 coffee farms (Jinotega)	1000	n/a	0.7%

Even when information was not available on what proportion of the sample was men, based on the occupational nature of the populations studied, it is likely that in all studies the proportion was very high. The prevalence rate in Jinotega was an order of magnitude lower than in the studies in Chinandega and Leon, which were all similar in prevalence. Although there is insufficient detail to determine whether there could have been quality problems in the Jinotega study, there are notable differences between the

two areas in geographic location (central vs. western), altitude, temperature, and type of crop, all of which could possibly affect the occurrence of CKD.

In the last two years, the Centro de Investigacion en Salud, Trabajo y Ambiente (CISTA) at the Universidad Nacional Autonoma de Nicaragua in Leon (UNAN-Leon) has conducted a number of random sample prevalence studies among 20-60 year old residents of selected communities in Leon and Chinandega. The first study was designed to determine prevalence among all residents of five different communities defined by the primary industry of employment (sugarcane/banana, mining, coffee, fishing, and services) (Torres, 2007). Eligibility criteria for the communities included: (1) location in Leon and Chinandega, (2) primary source of employment from one of the five aforementioned industries, and (3) population of 600-800 residents (except for coffee growing communities, all of which were smaller in size). From this list, one community within each industry was randomly selected.

Medical students from UNAN-Leon attempted to enroll all residents of the community 20-60 years of age. Study staff administered a questionnaire; measured blood pressure, height, and weight; and collected blood and urine samples. Serum creatinine was analyzed at the National Diagnostic and Reference Center laboratory at MINSA using an alkaline picrate rate-blanked compensated kinetic assay on a Roche Cobas Integra 400 analyzer (reported bias of 0.01 mg/dL versus a gold standard (IDMS) creatinine measurement). The reliability of the results was confirmed by the split sample method. CKD was determined by estimated glomerular filtration rate (eGFR) using the 4-variable MDRD Study equation (Levey, *Annals Int Med* 2001).

Similar methods were used in prevalence studies in the communities of Candelaria and La Isla in the municipality of Chichigalpa (Torres, 2008a) and in the remaining areas of Chichigalpa, categorized as urban or rural (Torres, 2008b). Candelaria and La Isla in particular have a very high concentration of residents who currently or formerly worked at ISA. The same is true to a lesser extent in other areas of Chichigalpa. In these studies, residences were randomly selected, and one resident per household between the ages of 20-60 was then randomly selected to participate. Finally, a similar study was conducted in five rural communities in northeast Leon (Aragon, 2009).

In addition, the Brookline-Quezalguaque Sister City Committee conducted a study based on the same protocol in Quezalguaque, which is located in the department of Leon between the municipalities of Leon and Chichigalpa (Brookline, 2008). All persons age  $\geq 18$  were eligible for the study. The 2007 census listing was used to randomly select 350 households stratified by "comarca", with the number of households proportional to the population of each comarca.

Medical and public health students from Boston University and Harvard University collected similar information and samples as in the UNAN-Leon CISTA studies from all household members in the eligible age range. However, serum creatinine was determined with the use of a portable device. The device measured the creatinine level and then calculated the eGFR using the MDRD Study equation only for individuals with

values below 60 ml/min per 1.73 m<sup>2</sup>. UNAN-Leon CISTA has since tested the device against the MINSA reference laboratory and found the results to be reliable (personal communication, Dr. Torres, 6/09). In the Brookline study, only persons with eGFR ≤60 ml/min per 1.73 m<sup>2</sup> were considered to have CKD. The creatinine assay used in the CISTA and Brookline studies were not calibrated to Cleveland Clinic or to IDMS traceable methods, which can introduce error in the eGFR. However, the impact is greatest at the lowest serum creatinine/highest eGFR values. Therefore the high rates of CKD Stage 4 disease observed (as described below) are not likely to be affected by differences in creatinine assay (Clase, 2002; Coresh, 2002; Stevens, 2007).

Despite their limitations, these studies provide the best data to date on the prevalence of CKD in the region. Furthermore, because they used random sampling methods with generally high response rates and were conducted using similar methods, they provide a basis for comparison. Table 7 shows response rates, sample sizes, CKD prevalence, and male:female CKD ratio for each of the 11 communities included in these studies. To permit comparisons between the two sets of studies, cases from the UNAN-Leon studies are reported based on eGFR ≤60 (Stages 3-5), and results from the Brookline study are reported only for participants ≤60 years old.

Overall response rates ranged from 73-94% (median 85%). The response rate among females was higher in all communities where sex-specific response rates were available, with differences ranging from 4-21%. To some degree, the lower response rate among men is due to the greater likelihood that they are away from the community or otherwise not at home during the times the study teams attempted to collect samples; in addition, men were more likely to refuse to participate than women. Total sample sizes ranged from 77-670 (median: 190).

Prevalence rates varied from 0% to 13.1% (median: 8.7%) and were above 8% in the sugarcane/banana and mining community, Candelaria, La Isla, urban Chichigalpa, and Quezalaguaque. Rates were lowest in the coffee (2.6%) and services (no cases identified) communities. However, because the proportion of males and females differs substantially across different communities—and in the absence of standardization—a sex-specific comparison is more appropriate. Among men, the rates by community ranged from 0-32.7% (median: 14.7%). Prevalence was >20% in La Isla, Candelaria, and urban Chichigalpa. With the exception of northeast Leon, men had a substantially higher prevalence rate than women, with ratios ranging from 3.1-38.1 and absolute differences of 5.0-28.9%. The largest absolute differences were in Candelaria (27.9%), La Isla (28.5%), and urban Chichigalpa (20.1%). For comparison, data from the US NHANES survey indicates that the prevalence of CKD stages 3 and 4 in the US population aged 20-59 years is 1.2% and the male to female ratio is 1.3 (Coresh J, 2005).

When results are further broken down by stage, the male:female ratio is much greater among more advanced cases (Table 8). Because of the different case definitions and how the data were categorized in available reports, the comparison in the UNAN-Leon CISTA studies is between Stage 1-2 (not included in the data reported in Table 7) and

3-5. For stages 1-2, we only have information from the studies conducted in Chichigalpa. In Quezalguaque, the ratio of men to women was 2:1 for Stage 3 and 13:1 for Stage 4-5. In the UNAN-Leon CISTA studies, with the exception of the study in northeast Leon, the male:female ratios were  $\leq 1.6$  for Stage 1-2 and ranged from 3:1 to 38:1 for Stage 3-5.

**Table 7. Response rates, sample sizes, and CKD prevalence in five community-based studies in Leon and Chinandega, 2007-2008**

	Response rate			Sample size			CKD prevalence (stage $\geq 3$ )			
<b>Torres, 2007: Residents of 5 communities in Leon and Chinandega</b>										
<i>Primary Industry</i>	T	M	F	T	M	F	T	M	F	M:F Ratio
Sugarcane/ Banana	86%	78%	94%	331	154	177	10.0%	17.5%	3.4%	5.1
Mining	86%	81%	89%	383	156	227	8.9%	14.7%	4.8%	3.1
Fishing	77%	69%	85%	166	91	75	6.0%	10.7%	2.2%	4.9
Coffee	84%	82%	86%	77	40	37	2.6%	5.0%	0.0%	n/a
Services	79%	67%	88%	140	49	91	0.0%	0.0%	0.0%	n/a
<b>Torres, 2008a, 2008b: Residents of different communities within Chichigalpa</b>										
Candelaria	94%			202	74	128	11.4%	29.7%	0.8%	38.1
La Isla	87%			175	55	120	13.1%	32.7%	4.2%	7.8
Other urban	74%			217	72	145	8.8%	22.2%	2.1%	10.7
Other rural				110	36	74	5.5%	16.7%	0.0%	n/a
<b>Aragon, 2009: Residents of 5 rural communities in northeast Leon</b>										
NE Leon	73%			190	72	118	6.3%	4.2%	7.6%	0.6
<b>Brookline, 2008: Residents of Quezalguaque, Leon</b>										
Quezalguaque	86%	76%	93%	670	255	415	8.7%	14.9%	4.8%	3.1

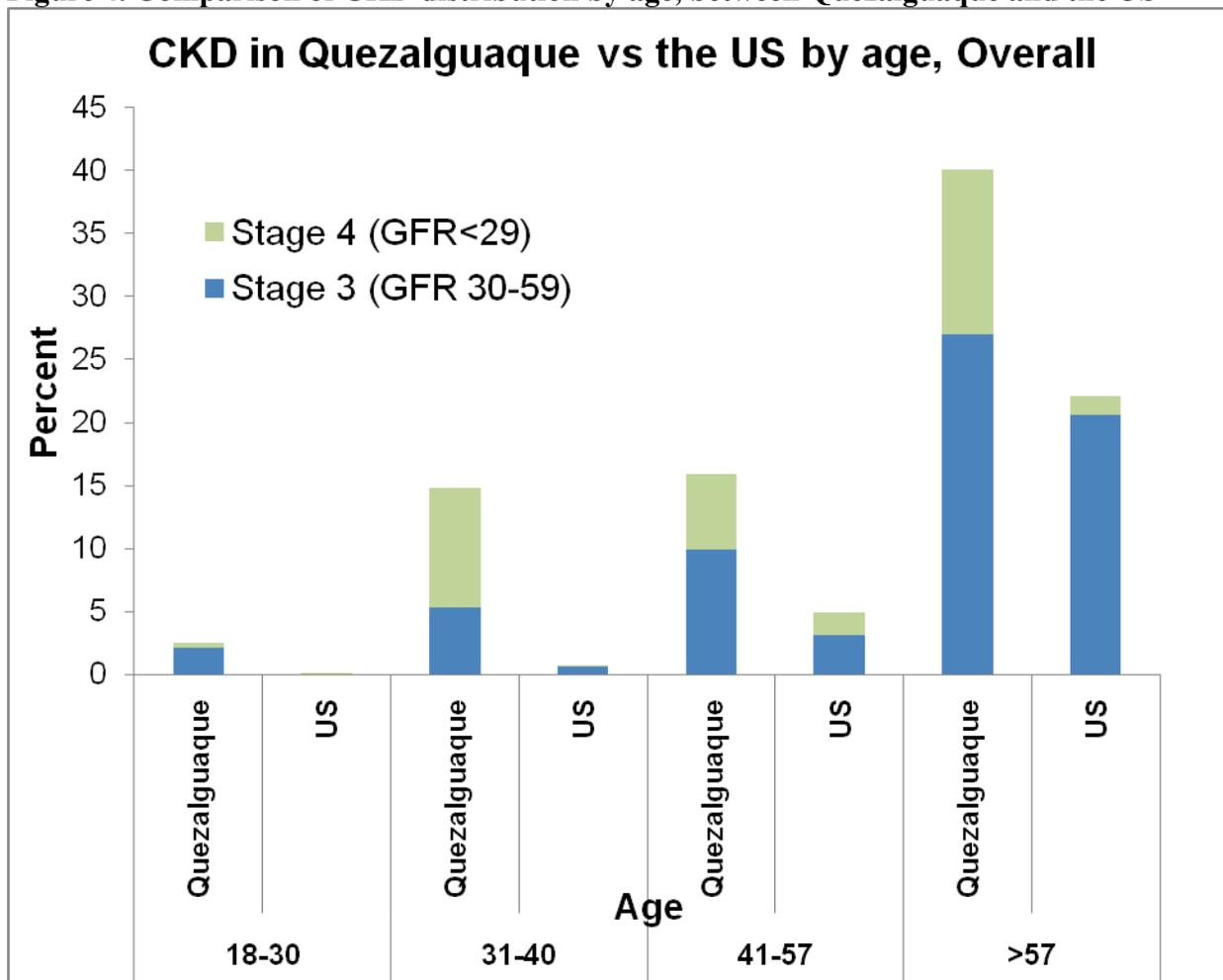
**Table 8. Sex ratios by CKD stage for studies conducted in Chichigalpa and Quezalguaque**

	Men	Women	Male:Female Ratio
<b>Candelaria</b>			
Stage 1-2	35.1	27.3	1.3
Stage 3-5	29.7	0.8	37.1
<b>La Isla</b>			
Stage 1-2	36.4	27.5	1.3
Stage 3-5	32.7	4.2	7.8
<b>Other Urban Chichigalpa</b>			
Stage 1-2	47.2	40.7	1.2
Stage 3-5	22.2	2.1	10.6
<b>Other Rural Chichigalpa</b>			
Stage 1-2	50.0	31.1	1.6
Stage 3-5	16.7	0.0	N/A
<b>Quezalguaque</b>			
Stage 3	8.6	4.3	2.0
Stage 4-5	6.3	0.5	12.6

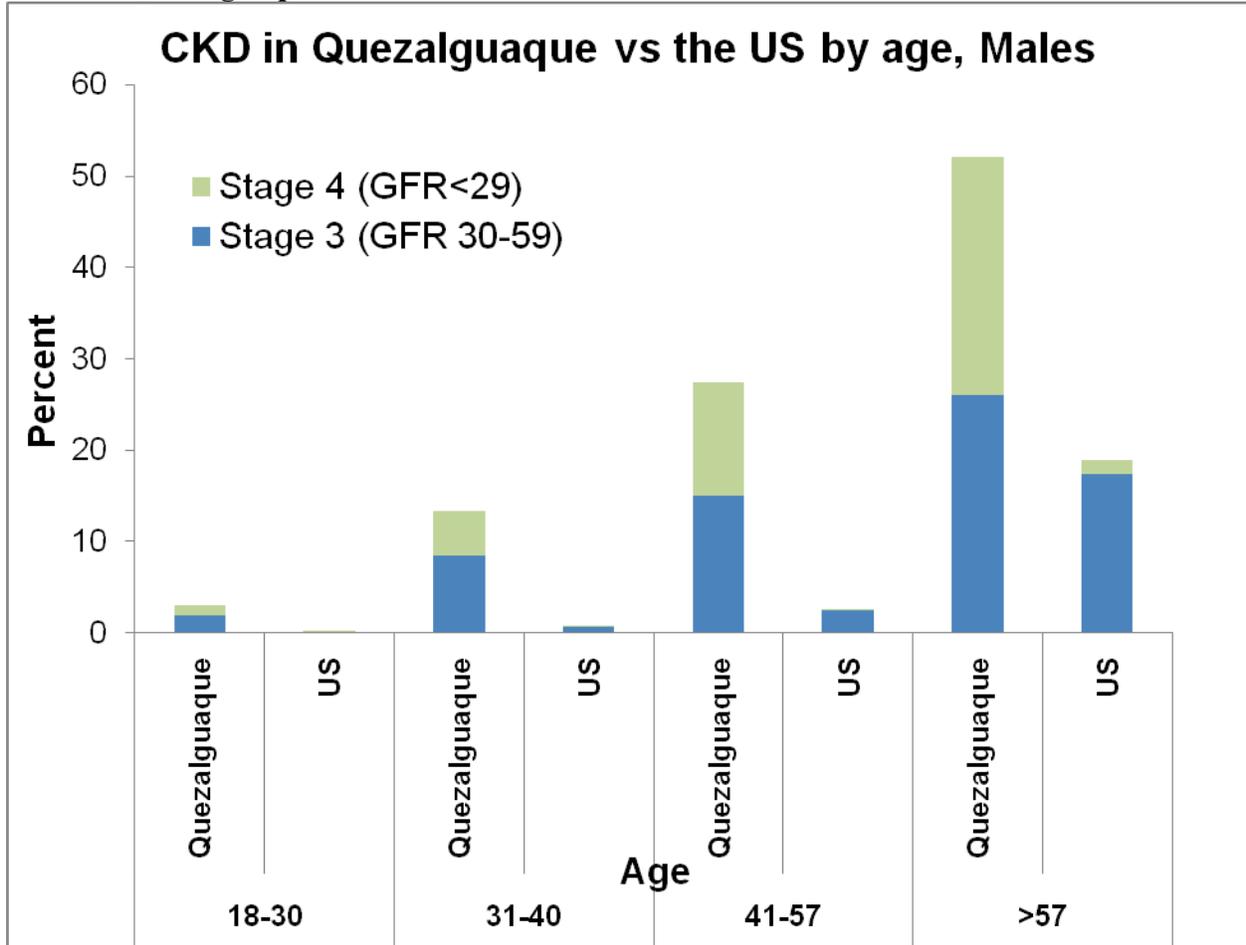
Figures 4-6 show data on CKD prevalence for Stages 3 and 4 from Quezalguaque and the U.S. National Health and Nutrition Examination Survey (NHANES) stratified by age

(18-30, 31-40, 41-57,  $\geq 57$ ) and sex. For this comparison with U.S. data, we included all participants in the Quezalguaque study rather than restrict the data to those age 20-60. Across both sexes combined, the age-specific prevalence of CKD is much higher in Quezalguaque compared to the U.S., and the differences are much more pronounced at both younger ages and higher stage. Sex-specific data demonstrate that this relationship holds for both men and women, but is much more pronounced among men.

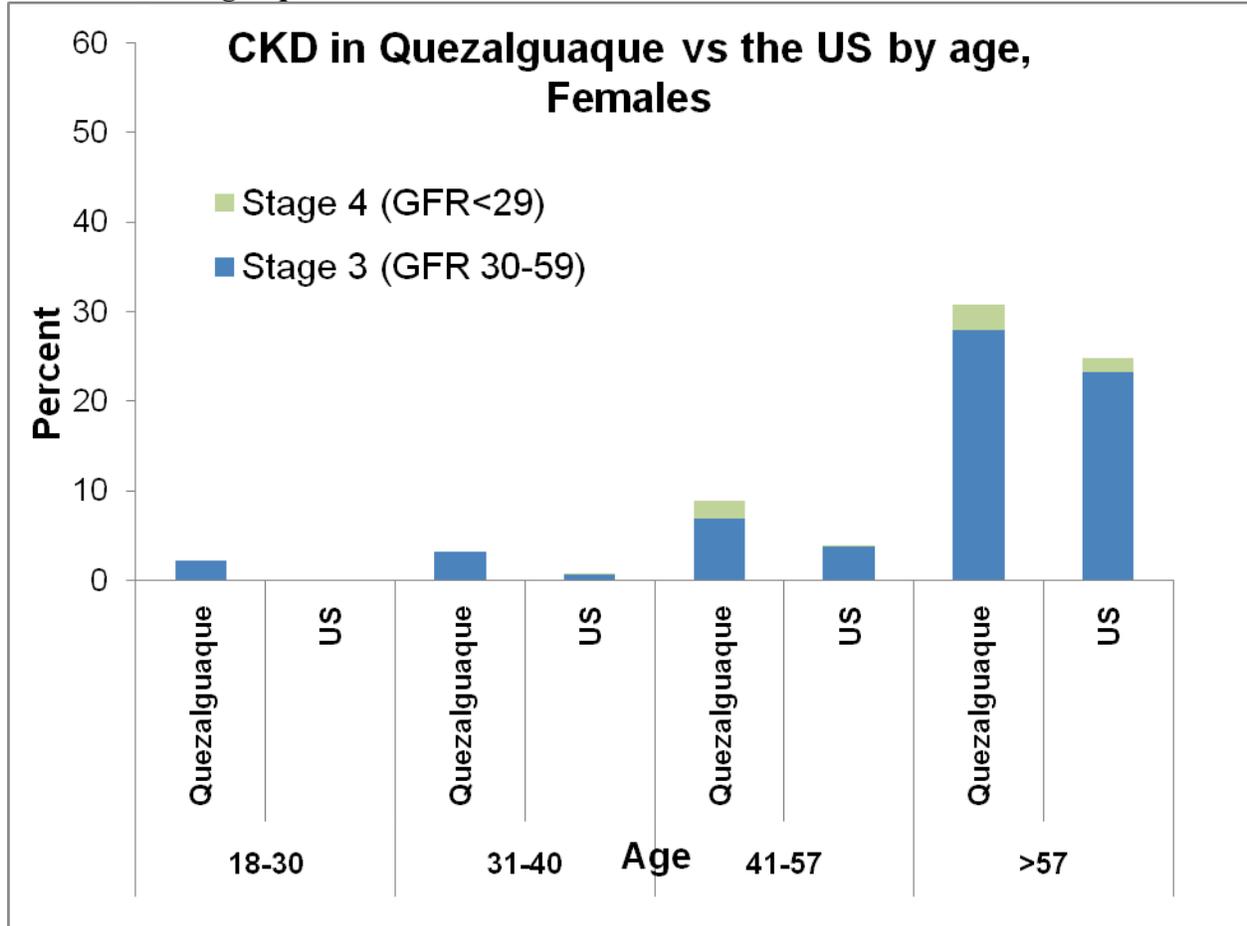
**Figure 4. Comparison of CKD distribution by age, between Quezalguaque and the US**



**Figure 5. Comparison of CKD distribution in males by age, between Quezalguaque and the US**



**Figure 6. Comparison of CKD distribution in females by age, between Quezalguaque and the US**



### 3. Interpretation of the data on CKD in Nicaragua

Because our primary concern is determining the etiology of CKD, incidence (defined as new cases) is the measure of greatest utility; however, the only available measures of CKD in Nicaragua are based on mortality and prevalence data. Under certain conditions, both measures can be used to estimate incidence rates, but at least some of those conditions are violated for both mortality (short survival time after occurrence) and prevalence (good estimate of average duration of survival after occurrence) of CKD in Nicaragua. However, we can use both measures to assess relative incidence under three conditions:

- (1) The extent of in-migration or out-migration from the population of interest is limited and not disproportionately concentrated among individuals with CKD or without CKD;
- (2) There are no important differences between the groups being compared in duration of survival after development of CKD; and
- (3) Among individuals who develop CKD, a high proportion die and a low proportion are

cured, and there is not much difference in these proportions between the groups being compared.

Based on our understanding, we believe that these conditions hold for the various comparisons of interest (e.g., variation by region, sex, occupation), so our interpretation of the data is stated in terms of "occurrence" (incidence) of CKD even though the underlying data are mortality and prevalence. These conditions do not hold for comparisons between Nicaragua and US data. We have not made comparisons between the two countries based on mortality data for both this reason and because of concerns regarding differences in the death registration systems. However, the methods to determine prevalence are much more similar, and differences in survival time after diagnosis would presumably inflate the prevalence in the US relative to Nicaragua; thus, the comparison is likely to be conservative. The record-based cohort study proposed in Section IV.A.4 should provide the first direct estimate of incidence rates among at least one group (sugar cane workers at ISA) in the population.

Based on the mortality statistics and prevalence studies described above, we have drawn several conclusions, which are detailed below along with a summary of the strength of evidence for each statement. We address the following below:

1. The occurrence of CKD is higher in the departments of Leon and Chinandega compared to other areas of Nicaragua.
2. The occurrence of CKD in the departments of Leon and Chinandega is higher among men than women.
3. The occurrence of CKD in the departments of Leon and Chinandega is elevated among younger age groups compared to other areas of Nicaragua and the U.S.
4. The occurrence of CKD is elevated among certain occupational groups compared to the general population.

*1. The occurrence of CKD is higher in the departments of Leon and Chinandega compared to other areas of Nicaragua.*

The data to support this conclusion are taken entirely from mortality statistics, which are the only source of national data on CKD in Nicaragua. Following is a consideration of the various biases associated with death certificates and their relevance to interpretation of the data:

a. Differential treatment: Mortality might be higher in one region, even though the prevalence is the same, if treatment is poorer in that region, leading more people with CKD to die. However, there is no indication that this is the case in Leon and Chinandega. In general, there is limited treatment available anywhere in the country for most people who develop CKD.

b. Differential attribution: As in the U.S., cause of death is determined by the physician. Autopsies are almost never conducted in Nicaragua, so the determination is based on

clinical information. Given the publicity that has surrounded the occurrence of CKD in Leon and Chinandega, one could plausibly argue that physicians in this area might be more likely to assign CKD as a cause of death than would physicians in other departments if presented with the same patient. However, mortality due to CKD was substantially higher in Leon and (especially) Chinandega in 1992, before there was any publicity (Table 5, Figure 2). In fact, the ratio of mortality rates for Chinandega compared to the country as a whole has not changed a great deal (3.2 in 1992 and 3.7 in 2005). In addition, while most of the controversy surrounding CKD in this region has been centered in Chinandega, Leon has seen a steeper increase in both absolute and relative terms than Chinandega and has increased substantially compared to the rest of the country (ratio of 2.0 in 1992 and 4.5 in 2005).

c. Differential completeness of registration: As with many developing countries, Nicaragua has underregistration of death certificates, reported to be 42% for the years 1950-1999 (<http://www.scielosp.org/img/revistas/bwho/v83n3/html/a09tab02.htm>). Even in the absence of differential attribution, if registration were more complete in Leon and Chinandega, this would artificially raise the mortality rate compared to other departments. However, the higher rate should be seen not only for CKD but generally for other causes of death as well. Although such data undoubtedly exist, we have not obtained them as of this time.

d. Differential accuracy of population denominator: The denominator of the mortality statistics are taken from the national censuses, which have been conducted in 1971, 1995, and 2005. Except perhaps for the 1992 data (which may have used the 1971 or 1995 Census), all the other years (1997, 2002, 2005) used data from the same general time period, so subsequent shifts in population are unlikely to have caused bias in the denominator. If the census were more likely to undercount the population in Leon and Chinandega than in other departments, this would lead to an inflation in the rate relative to other departments. Again, inflated rates should be seen for overall mortality and across a variety of conditions.

e. Differential migration between residence of incidence and residence at death: As in the U.S., deaths are attributed to the residence at time of death, which can be different than the residence at time of developing the condition. If a substantial number of individuals who developed CKD elsewhere migrated to Leon and Chinandega and died there, this would inflate the apparent rate. We have no evidence that either supports or conflicts with this hypothesis. We do note that one motivation for migration (better treatment) is not likely to be the case even today, and was certainly not true in the 1990s when the rates were already higher in Leon and Chinandega.

f. Different age structure: It is not clear whether the data are age-adjusted. If Leon and Chinandega have an older age structure than the rest of the country, the crude mortality rate would be inflated. We have no direct information on age structure, but the fact that the 2005 age-specific mortality rates are also highest in Leon and Chinandega suggests that this is not an explanation for higher rates (Table 9).

**Table 9. Age-specific mortality rates for CRI by department, Nicaragua 2005**

<b>Tasa de Mortalidad por IRC (10 000 h) de acuerdo a grupos etareos, Nicaragua 2005</b>					
	5 a 14	15 a 34	35 a 49	50 y más	Total
Boaco	0.00	0.00	0.95	2.21	0.40
Carazo	0.00	0.33	0.78	3.87	0.78
Chinandega	0.10	1.46	9.50	17.70	4.12
Chontales	0.00	0.00	0.89	2.21	0.39
Estelí	0.00	0.00	0.99	1.59	0.35
Granada	0.00	0.49	3.16	7.93	1.72
Jinotega	0.00	0.09	0.00	1.03	0.12
León	0.12	1.06	7.84	25.38	5.03
Madríz	0.00	0.00	0.00	0.64	0.08
Managua	0.00	0.26	0.47	3.60	0.85
Masaya	0.14	0.19	0.45	1.95	0.41
Matagalpa	0.08	0.18	0.32	2.24	0.36
Nueva Segovia	0.00	0.13	0.00	0.46	0.10
RAAN	0.00	0.00	0.00	1.20	0.10
RAAS	0.00	0.09	0.00	1.11	0.13
Río San Juan	0.00	0.00	0.00	1.13	0.10
Rivas	0.00	0.00	1.66	3.99	0.83
<b>Nicaragua</b>	<b>0.03</b>	<b>0.29</b>	<b>1.92</b>	<b>6.06</b>	<b>1.09</b>

Fuente: MINSA Mortalidad por IRC 2 005, INEC, Informe Censo 2005, Población por grupos etáreos

Absent a national prevalence study, or one which at least encompasses both high and low mortality departments, we can not absolutely rule out the possibility that the observed elevation in mortality rates in Leon and Chinandega can be attributed to one or more of the these biases rather than a truly higher occurrence of CKD. However, given the very large differences in mortality rates between Leon and Chinandega and other departments, it is difficult to imagine that these biases could be operating to such an extent as to explain even the majority of the excess.

Secondary evidence supporting the particularly elevated rate in this region includes the high rate of admissions for CKD to the regional hospital (Hospital Espana) in Chinandega (personal communication, Dr. Roberto Fernandez, Director, 6/09) and the

existence of a separate unit dedicated solely to diagnosing and treating patients with CKD at the local health center (Centro de Salud) in Chichigalpa (personal communication, Dr. Erwin Reyes, nephrologist, 6/09), where the staff includes one of the few nephrologists in Nicaragua.

We also make the following secondary points:

1. Some of the biases discussed above are more sensitive to changes across time. Therefore, we have not drawn any conclusion regarding whether the mortality rate is truly increasing over time or is simply coming to greater awareness and attention.
2. Although not as high as in Leon and Chinandega, elevated rates are also seen in other departments in the western zone in the Pacific region.

*2. The occurrence of CKD in the departments of Leon and Chinandega is higher among men than women.*

The evidence for this relationship is even stronger than for the overall excess in Leon and Chinandega compared to other departments, because the evidence is consistent from both mortality data and random prevalence studies. At the same time, one study demonstrated a rate that was similar or higher in women than men among residents of rural communities in northeast Leon (Aragon, 2008), suggesting that the increased prevalence of men may not hold true in all places.

The prevalence studies also indicate that men have more severe disease than women. If this is the case, it could be another contributing factor to the higher mortality rate in addition to higher prevalence, and also suggests the possibility of a different etiology or different factors affecting progression.

*3. The occurrence of CKD is higher among younger age groups in the departments of Leon and Chinandega compared to other regions of Nicaragua and the U.S.*

The age-specific mortality data in Table 9 show that mortality rates are 4-5x higher than rates for Nicaragua as a whole among the 15-34 and 35-49 age groups and 3-4 times for the  $\geq 50$  age group, and 2-3x higher in each age group than the next highest department. Although all the same concerns about death certificate data are relevant, the same arguments advanced in relation to the overall CKD-specific death rate also hold for the age-specific strata.

It is not appropriate to compare death rates between Nicaragua and the U.S. as a proxy for incidence because death due to CKD generally occurs much later after diagnosis in the U.S. due to the availability of effective treatments. Therefore, the prevalence data from Quezalguaque provides the best evidence for higher age-specific rates in at least one town in Leon compared to the U.S. Data from those UNAN-Leon CISTA studies for which we were able to identify age-specific rates (Candelaria, La Isla, and urban Chichigalpa) also showed elevated rates among younger age groups, particularly among men (data not shown).

Secondary evidence supporting a young age at occurrence comes from data collected by ASOCHIVIDA from 930 members. Among the 618 providing data (99.3% male) on the age they were no longer able to work at ISA due to having a high creatinine level ( $\geq 1.2$  mg/dl), the mean and median were 43 and the interquartile range (25-75%) was 34-51, indicating that 25% had a high creatinine level by their early 30s.

*4. The occurrence of CKD is elevated among certain occupational groups compared to the general population.*

Given their disproportionate incidence of CKD and representation in the occupations of primary interest, we use data mainly from males in this section.

The five-community study in Leon and Chinandega by UNAN-Leon CISTA shows a clear differentiation among males according to community, with the highest prevalence rates found in the two communities where sugar cane/banana cultivation and mining were the primary economic activity (Torres, 2007). The fishing community also had relatively high prevalence, while communities whose economies centered primarily on coffee and services had lower rates.

Other evidence supports these results. The two communities with the highest mortality rate in the country (approximately 50% higher than the next highest community and 12 times higher than the national rate) were Chichigalpa and Larreynaga. The main economic activity in the former community is sugar cane cultivation and in the latter community is mining. In addition, the study by Siqueira (2003) among coffee workers, although conducted in the neighboring department of Jinotega, also found a very low prevalence of elevated creatinine.

One limitation of the five-community study is that the communities of residence are taken to represent the respective occupations. Although likely a good proxy measure, we have no data regarding the percentage of the population employed in the primary industries. Information on usual occupation of the study participants was collected, but data have not yet been presented in that form.

Taken together, the two studies in Chichigalpa also provide evidence that sugar cane workers have an elevated occurrence of CKD (Torres, 2008a; Torres, 2008b). The areas of Candelaria and La Isla have a very high proportion of current and former sugar cane workers (in fact, Candelaria was created to house the workers who lived on the ISA grounds prior to 2000). The prevalence of CKD among males was very similar in these two communities (~30%), which is almost 80% greater than the next highest of the communities outside Chichigalpa listed in table 7 where banana and sugar cane cultivation are the main activities (18%) and twice as high as in the mining community. Furthermore, in urban Chichigalpa, which has a wider range of occupations, 27% of participants had worked in sugar cane and had a prevalence ratio of 5.9 compared to those who had not worked in sugar cane (data not shown, Torres 2008b). Therefore, although the overall prevalence was 22%, the prevalence among residents with a history of working in sugar cane was substantially higher than 22% and prevalence

among other residents had to be lower. A similar relationship was seen in rural Chichigalpa.

Other facts need to be considered which temper the previous paragraph's focus on elevated prevalence among sugar cane workers:

(1) The comparisons between sugar cane workers in Chichigalpa and other communities can be based only on the small number of sites where studies have been conducted. While we can conclude that the prevalence in Chichigalpa is quite elevated, we cannot therefore conclude that it must be higher than all other communities in the region.

(2) The two studies by Callejas Callejas et al. (2003a, 2003b), using the creatinine of >1.5 mg/dl as a case definition, found not dissimilar prevalence for sugar cane workers at another ingenio (10%) and workers living in non-sugar cane areas without a history of working in sugar cane (7%). However, the fact that the latter study was based on a sample of interested volunteers raises concerns about possible bias and makes it difficult to compare to the sugar cane worker-based study.

(3) As previously mentioned, the mining community of Larreynaga in Leon has as high a CKD mortality rate as Chichigalpa.

(4) Although we do not have data on the proportion of males in Leon and Chinandega who have worked in sugar cane, geography would suggest that the percentage is higher in Chinandega based on the historical and current presence of two sugar cane companies. Nevertheless, since 1997, the department of Leon has had as high a CKD mortality rate as Chinandega, and was actually higher in 2005.

Based on consideration of all the evidence, we believe the most appropriate interpretation of the data is:

(1) There is a wide variation in the prevalence of CKD by occupational group in the region.

(2) Sugar cane workers are one of the occupational groups with a high prevalence of CKD.

(3) Sugar cane workers are not unique in having a high prevalence of CKD

In summary, national mortality statistics and prevalence studies conducted almost exclusively in Leon and Chinandega do provide data sufficient to characterize the basic epidemiologic characteristics of CKD in these two departments. The evidence is strongest that CKD is more common in men than women in Leon and Chinandega. This fact alone is a powerful clue as to etiology, because any identified cause(s) should be consistent with this observation. There is also quite strong evidence that CKD is more common in Leon and Chinandega than other areas of the country and that sugar cane workers in this region have elevated rates. It is important to note that this latter fact does not necessarily mean that occupational exposures must be the cause of CKD. However, it does mean that an occupational etiology -- either singly or contributory -- is a plausible hypothesis that needs to be addressed. It is also very likely that other occupational groups are likely to have elevated rates. We have concluded that the evidence is stronger for sugar cane workers, not because we think elevated rates of CKD are likely

to be unique among this group, but simply because they have been studied more often. In addition, we have not concluded that sugar cane workers have higher rates of CKD than all other occupations, simply that the rates are elevated. There may be quite a number of other occupations among which rates are as high as among sugar cane workers but which have not yet been studied.

## **4. Summary of Prior Research on CKD in Nicaragua**

### **a. Overview**

While descriptive statistics and case series are useful for assessing the impact of CKD on the Nicaraguan population and for providing clues about etiological hypothesis, they cannot be used to test these hypotheses mainly because they do not have explicit comparison groups and do not take extraneous confounding factors into account. Thus, analytical studies, such as case-control, cross-sectional, and other designs, must be conducted to determine if a valid epidemiological association is present between CKD and a particular exposure.

Therefore, in addition to the data on mortality and prevalence described in the previous section, we also reviewed a number of studies that tested hypotheses about putative causes of CKD in Nicaragua from documents already in our possession, accessed through CAO, or obtained directly from investigators in Nicaragua. As described later, due to their limitations (many of them unavoidable), most of these studies have better served as a means for screening hypotheses rather than testing them. We identified 22 unique analytical studies on CKD in this region, including 21 case-control and cross-sectional studies and one experimental study.

The key findings and limitations of these studies are summarized below.

### **b. Case-Control and Cross-sectional Studies**

One of the main designs in epidemiological research, case-control studies are particularly useful in settings where little is known about the causes of a disease and when the disease appears to have a long induction period, both of which appear to be true for CKD in Nicaragua. In this type of study, the investigator identifies and enrolls cases of particular disease and identifies and enrolls a control group which represents sample of the population that produced the cases. Then the investigator determines and compares the exposure history in the two groups to assess whether there is association between prior exposure and disease. Typically many different exposures are examined and care is taken to avoid biases and to control for extraneous confounding variables. Like case-control studies, cross-sectional studies are also able to examine the association between a disease and an exposure; however, cross-sectional study populations are typically selected without regard to exposure or disease status and examine the current prevalence of an exposure in relation to the current prevalence of a disease. Thus, cross-sectional studies often have unclear temporal relationships.

Upon review of the available documents, we identified 21 unique case-control or cross-sectional studies conducted on CKD in Central America, with 19 of these conducted in

Nicaragua. One was conducted in four other Central American countries along the Pacific coast, and another was conducted in El Salvador. All but one of these studies were conducted by local investigators; one was conducted by U.S. investigators from the Brookline Quezalguaque Sister City Committee. The documentation of the study methods and results was quite variable. Studies with good documentation tended to be research theses conducted by medical students. Studies with poor documentation tended to be described only in PowerPoint presentations. To the best of our knowledge, only one study (Garcia-Trabanino, 2004) has been published to date in a medical journal.

Most of the studies were conducted in community settings; only three were conducted in hospital settings. Among the latter, cases generally had more “severe” CKD than those in community-based studies, and controls were patients hospitalized for other reasons. The number of CKD cases in the studies ranged widely from 15 to 600 but the case definitions were fairly uniform: cases were generally defined either by a serum creatinine above a certain level (e.g. serum creatinine  $\geq 1.5$  mg/dl) or by a glomerular filtration rate less than a certain level (e.g. GFR  $< 60$  ml/min per  $1.73$  m<sup>2</sup>). Personal interviews were the main data collection technique for all of the studies. A few studies collected biological samples for exposure analysis; however, it appears that some of these analyses have not yet been conducted.

#### **i. Case-Control and Cross-sectional Study Results**

As described in Tables 10a-k and the following paragraphs, the results of the case-control and cross-sectional studies provide results on wide variety of putative causes of CKD, including exposure to certain occupations (generally defined as either agricultural or sugar cane work), heavy metals, and pesticides; medical conditions including dehydration, urinary tract infections, diabetes, and hypertension; use of non-steroidal anti-inflammatory drugs; lija and alcohol consumption; cigarette smoking, and family history of kidney disease. The level of evidence for particular exposure is quite variable. For example, only one study provides results on cadmium exposure while 17 studies provide evidence on lija or alcohol consumption. One community-based study was excluded from this review because the results could not be interpreted (Cooper Mendoza, 2008).

##### **i. a. Occupation**

Thirteen studies have examined occupation in relation to CKD (Table 10a). These studies focused mainly on agricultural work, and in some instances, on work characteristics such as duration of employment and crop type, and 12 of these studies report modest to strong associations. Odds ratios for agricultural work ranged from 1.6 to 4.2, while even stronger associations were seen for work in sugar cane cultivation, particularly for long durations (e.g., Odds Ratios=6.0-12.7). The few studies that examined other types of work found increased risks for fishing (Odds Ratio=4.5), mining (Odds Ratio=1.8), cotton cultivation (Odds Ratio=2.8), and banana cultivation (Odds Ratio=4.7). Many of the occupation findings were statistically significant. The only study with null findings was the Sister City Study conducted in Quezalguaque. Only a few of

these studies controlled for confounding variables. Results of three of the four studies that controlled for at least age and sex found modest to strong associations for agricultural work (Odds Ratios: 1.6-5.9) (Garcia-Trabanino, 2004; Castrillo, 2001; Torres Gonzalez, 2007). The fourth study (from Quezalguaque) was null (Brookline Sister City Health Committee, 2008).

#### i. b. Pesticides

Nine of the 13 studies that examined pesticide exposure found modest to strong associations (Table 10b). Odds ratios ranged from 1.6 to 9.3 and most were statistically significant. Exposures to specific pesticides were not examined. Only a few of these studies controlled for confounding variables. The three of the four studies that controlled for at least age and sex found 1.9 to 2.6-fold increased risks (Castrillo, 2001; Lopez Arteaga, 2005; Brookline Sister City Health Committee, 2008). The fourth study conducted in El Salvador was null (Garcia-Trabanino, 2004). The latter included agricultural work as a confounder in the multivariate analysis and so may have masked a possible association with pesticide exposure.

#### i. c. Heavy Metals: Lead and Cadmium

Five epidemiological studies examined the relationship between lead and CKD, with inconsistent results (Table 10c). One study found a very strong association (Odds Ratio: 18.9) (Marin, 2002). While no measure of statistical stability was reported in this study, the sample size was small. Another study found high but similar lead levels among cases and controls. Confounding was inadequately addressed in these two studies. In addition, the Brookline Sister City Study found only one of 771 community members with high blood lead levels and this individual had a normal GFR. The two remaining studies did not report lead levels comparing cases and controls.

To date, only one small case-control study has examined cadmium exposure in relation to CKD (Table 10a, Uriarte Barrera, 2000). Estimated levels were higher among 15 cases versus 15 controls (0.73 versus 0.42 ug/day) but the difference appears to be related to differences in cigarette smoking. Another case-control study has reportedly collected hair samples for heavy metal analyses but no cadmium results were found in available documents.

#### i. d. Dehydration

Six studies examined some aspect of the physical environment (e.g., high temperature) or symptoms (e.g., thirst) related to dehydration (Table 10d). Four of the studies found strong positive associations (Odds Ratios=1.8-3.9 for thirst and 4.2 for high temperatures) while two did not (Odds Ratios 0.2-1.2 for thirst). A seventh study, which compared electrolyte consumption among workers who became ill versus those who did not, found much lower consumption among ill workers. Again, only a few studies controlled for confounding variables. The only study that controlled for age and sex found a 1.8-fold increased risk for thirst (95% CI: 1.0-3.6) (Brookline Sister City Health Committee, 2008).

#### i. e. Urinary Tract and Kidney Infections

Ten studies examined a history of urinary tract or kidney infections (Table 10e). In some studies exposure was defined as recurrent infections. The results of these studies were inconsistent; six found modest to large positive associations (Odds Ratios=1.4-5.4) while four had essentially null findings. Three of the six positive studies had statistically significant findings. However, only a few of the studies controlled for confounding variables. The two studies that controlled for at least sex and age found 1.4-fold and 3.2-fold increased risks (Castrillo, 2001; Lopez Arteaga, 2005).

#### i. f. Diabetes

Fifteen studies examined a history of diabetes (Table 10f). The results of these studies are inconsistent; six found modest to large positive associations (Odds Ratios=1.8-3.5) while the remainder had null findings. The prevalence of diabetes was low and so most results were statistically unstable. In addition, only a few of these studies controlled for confounding variables. The five studies that controlled for at least sex and age also had inconsistent results (adjusted Odds Ratios=0.7-2.1) (Garcia-Trabanino, 2004; Castrillo, 2001; Torres Gonzalez, 2007; Lopez Arteaga, 2005; Brookline Sister City Health Committee, 2008).

#### i. g. Hypertension

Fourteen studies examined a history of hypertension (Table 10g). Ten of the studies found modest to strong positive associations (Odds Ratios=1.7-6.3); the strong associations tended to be statistically significant. The remaining four studies had null findings. In addition, only a few of these studies controlled for confounding variables. The four of the five studies that controlled for at least sex and age found small to large positive associations (Odds Ratios 1.4-6.3) (Garcia-Trabanino, 2004; Castrillo, 2001; Torres Gonzalez, 2007; Brookline Sister City Health Committee, 2008).

#### i. h. Non-Steroidal Anti-inflammatory Drugs

Ten studies examined the use of non-steroidal anti-inflammatory drugs (NSAIDs, Table 10h). Only two of the studies found positive associations that were statistically significant (Odds Ratios=4.0-4.2), while the remainder had essentially null findings. The two studies that controlled for at least sex and age found adjusted odds ratios of 1.1 and 1.2 (Lopez Arteaga, 2005; Brookline Sister City Health Committee, 2008).

#### i. i. Lija and Alcoholic Beverage Consumption

Seventeen studies examined the consumption of alcoholic beverages, including lija (Table 10i). All of the studies that reported on lija consumption (n=6) found moderate to strong positive associations (Odds Ratios=2.0-11.0) that, when reported, were statistically significant. The studies that reported on alcohol consumption were less consistent; eight reported modest to strong positive associations (Odds Ratios=1.4-7.6) while six reported essentially null findings. In addition, only a few studies controlled for confounding variables. The single study that appears to be well-controlled found an adjusted odds ratio of 1.4 (95% CI: 1.1-4.8) for alcohol consumption among male workers (Lopez Arteaga, 2005).

#### i. j. Cigarette Smoking

Six studies examined cigarette smoking (Table 10j). The two studies with positive associations found 1.8 and 3.8-fold increased risks and the latter was statistically significant. The remaining four had essentially null results. Only a few of these studies controlled for confounding variables. The results of the Brookline Sister City Study, which controlled for age and sex, were null (2008).

#### i. k. Family History

Eleven studies examined family history chronic kidney disease or renal failure (Table 10k). Seven of these studies found modest to strong positive associations with odds ratios ranging from 1.6 to 4.0; many of these findings were statistically significant. The remaining four study results were essentially null. Only a few of these studies controlled for confounding variables. The single study that appears to be well-controlled found an adjusted odds ratio of 1.6 (95% CI: 1.1-2.4) for a family history of chronic kidney disease (Lopez Arteaga, 2005).

#### i. l. Miscellaneous Exposures

Two studies reported some interesting results for exposures not listed above (Table 10l). Ruguma (2001) reported that glucosamine use was associated with a 3.7-fold increased risk of CKD (95% CI: 2.2-6.3). In addition, Aragon et al. reported that dengue infection was associated with a 2.7-fold increased risk (95% CI: 0.8-9.1). Confounders were not adequately controlled in these investigations.

### **c. Experimental Study**

In addition to the case-control and cross-sectional studies, we also identified one experimental study. Its purpose was to determine the impact of preventive measures to avoid deterioration of renal function due to heat stroke syndrome among field workers in the San Antonio sugar mill in western Nicaragua during 2005-2006 (Solis Zepeda, 2007). The study, which compared 218 workers who received education about the consumption of electrolyte solutions and energy cookies in the field versus 187 workers who did not, found that these preventive measures had a small, beneficial impact on kidney function.

### **d. Summary of Results of Prior Epidemiological Studies**

The above described studies provide results on wide range of putative causes of CKD. These studies reported fairly consistent positive associations for agricultural work, pesticide exposure, dehydration, hypertension, lija consumption, and family history of CKD. Positive associations were observed for these exposures even among the few studies that controlled for confounding variables (at least age and sex). Results for the remaining exposures were either inconsistent or essentially null. As described below, interpretation of this body of research must consider a variety of limitations.

### **e. Limitations of Prior Epidemiological Studies**

While 22 distinct epidemiological studies have been conducted on CKD in Nicaragua and nearby countries, the validity of their findings is difficult to assess for the following

reasons. First, we often did not have access to complete descriptions of the study methods. Thus, it was challenging to determine, for example, if bias was present or if confounding was adequately controlled. However, among studies with sufficient description, we identified several limitations that make their interpretation problematic. These include uncontrolled confounding, recall bias from the use of interviews to collect retrospective exposure data, failure to consider synergistic effects of two or more risk factors, misclassification of exposure information, and low statistical power stemming from a small number of subjects. Some of these problems (e.g., recall bias, uncontrolled confounding) would tend to bias the results away from the null making associations appear stronger than they are, while others (e.g., misclassification and low power) would tend to bias the results towards the null thereby masking associations.

One of the most concerning aspects of this body of research is uncontrolled confounding. Confounding means that the association is invalid because there is a mixing of effects between the exposure, disease and a third extraneous variable known as a confounder. Evidence for uncontrolled confounding among the reviewed studies includes the failure to control any confounders in some studies, controlling for only a limited number of confounders in most others with little or no justification for controlling certain confounders while omitting others. We believe that this issue is particularly problematic for exposures that are closely related to one another. For example, separate strong associations were reported for two related exposures --history urinary tract infections and the use of NSAIDs; however, analyses examining one exposure did not control for the other and so it is impossible to determine if these associations are valid or if they confound one another.

Another important problem is recall bias which stems from the use of interviews to collect retrospective exposure data. Recall bias occurs when there is a differential level of accuracy in the information provided by the compared groups (e.g. cases and controls). The classical recall bias scenario whereby diseased cases have *more* accurate recall of exposures than the controls is a concern among the Nicaraguan studies because there widespread knowledge of the CKD problem and it is likely that many study subjects already had strong ideas about its causes. In particular, a study of workers from Ingenio San Antonio (Zelaya, 2001) found strong positive associations for most exposures that were examined. This is a highly unusual occurrence in an epidemiological study and raises the suspicion of recall bias. In fact, many of the exposures, such as working in high temperatures, were likely present for both cases and controls, and so null associations would be expected.

Another drawback of the prior studies is their failure to take into account the impact of two or more factors simultaneously that may be working in concert to produce CKD. In other words, there may be a synergistic relationship between two or more exposures that increase risk of disease beyond what we would expect from simply adding the risks associated with each factor alone. For example, volume depletion may make the kidneys more susceptible to the effects of other exposures such as heavy metals and NSAID use. While each factor alone may lead to a modest increase in risk, the

combination of both factors may create “the perfect storm” and lead to a large increase in risk.

Still another limitation is exposure misclassification, which is one of the most common problems in epidemiological research. This problem can arise when broad categories are used to classify exposure. For example, some studies defined the exposure as “agricultural work” or as “pesticide exposure,” even though it is likely that only certain types or aspects of agricultural work and that only certain types of pesticides increase the risk of CKD. While broad exposure classifications give a general idea of a putative cause, they make it difficult to identify effective preventive measures and tend to bias results towards the null. Misclassification can also arise for “clinical” exposures. For example, true urinary tract infections are uncommon in males, yet this condition was frequently reported among male subjects, particularly those with CKD.

Last but not least, none of the existing studies test other hypotheses regarding the causes of CKD in Nicaragua, including exposure to aristolochic acid (known to cause CKD in the Balkans); known infectious diseases; and the use of nephrotoxic antibiotics and other drugs.

In summary, the studies previously described in this section provide results on a wide range of putative causes of CKD. Taken together, these studies reported fairly consistent positive associations for (1) agricultural work, (2) pesticide exposure, (3) dehydration, (4) hypertension, (5) lija consumption, and (6) family history of CKD. Positive associations were observed for these six exposures even among the few studies that controlled for confounding variables (including age and sex). Results for the remaining exposures were either inconsistent or essentially null. Because the positive findings were relatively consistent and some confounders were controlled, we have slightly more confidence in their validity. However, as noted above, all of the prior studies were questionnaire-based, and so we cannot rule out the possibility that recall bias (as well as other problems) accounts for the findings. Thus, as described in greater detail in Section IV, we recommend that an entirely different approach be taken for future studies of CKD in Nicaragua. Instead of questionnaires, our recommended approach includes environmental sampling, analysis of biological samples, work observation and a record-based cohort study.

**Table 10a Occupation**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
6	Garcia-Trabanino, Dominguez, Jansa, Oliver 2004	Cross-sectional	Two communities in El Salvador: 1 in coastal and 1 in high elevation area	291 male volunteers from coastal region; 62 male volunteers from control community	Cases=Proteinuria and CR >1.4	Hypertension, diabetes, age, region, alcohol use	OR=1.6 (0.8-3.5) for agricultural work
26	Castrillo, Bonilla, Estrada	Case-Control	Hospital-based in Leon	65 cases and 130 controls	Cases=CKD symptoms and CR >1.2	Sex and age matching	OR=3.8 (1.4-10.8) for agricultural work

	2001						
40	Marin Ruiz, Berroteran 2002	Case-Control	Workers from Ingenio San Antonio	43 cases, 39 controls	Cases= workers with CKD, Controls=workers without CKD	Unknown	OR=6.0 for working more than 15 years, OR=13.3 for working more than 8 hours a day
56-57	Torres, Gonzalez, Aragon, Wesseling, Lundberg 2007	Cross-sectional	Five northwestern communities with working populations: mine workers, agricultural workers; service workers; fishermen; coffee growers	479 women and 617 men	Cases=CR >1.2 for males and 0.9 for females	Age and sex	Among males mining (OR=5.9), banana/sugar cane work (OR=5.9), and fishing (OR=4.5) were associated with highest relative risks; Among females, mining (OR=1.8) was associated with the highest relative risk. Service workers had reduced relative risks (OR=0.3 in men and OR=0.5 in women).
58	Torres, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in Chichigalpa	327 subjects	Stages 3 and 4: GFR <=60; n=159	Stratification by location of residence (rural/urban)	OR=5.9 (p<.01) for current cane work among urban subjects and OR=15 (p<.01) for cane work among rural subjects
59	Torres Lacourt, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in La Isla and Candelaria	175 in La Isla and 202 in Candelaria (94-95% participation rates; 38-43% of population)	Stages 3 and 4: GFR <=60 n=76 in La Isla and 84 in Candelaria	No	OR=4.6 (1.8-11.8) for cane work in La Isla and OR=12.7 (4.5-36) for cane work in Candelaria, OR=2.2 (1.0-4.7) for cotton work in La Isla; OR=3.7 (1.5-8.9) for banana work in La Candelaria
67	Lopez Arteaga 2005	Case-Control	Community-based in Leon and Chinandega	490 cases and 3320 controls	Cases= CR>1.3 and GFR <80; Controls=CR <1.3 and GFR >80	Only male workers included; Conducted logistic regression which controlled for other types of agricultural work	Strongest ORs for cotton (OR=2.8); sugar cane (OR=2.4); rice (OR=1.5) and corn (OR=1.4)
	Alonso Medrano, Perea 2002	Case-control	Sugar cane workers in Chichigalpa	44 cases 100 controls	Cases: workers since 1999 with symptoms and CR>=2.5; Controls: active workers without symptoms and CR<1.1	None	Average duration of cutting cane=15 years for cases and 6.7 years for controls
	Callejas Callejas, Alonso Medrano, Mendoza 2003	Case-Control	Hospital-based in El Viejo, Chinandega. All sugar cane workers.	38 cases 111 controls	Case=CR>=1.5	Certain analyses	OR=2.9 for cutting sugar cane > 10 years (crude)

	Sister City Health Committee 2008	Case-Control	Community-based in Quezalguaque	95 cases and 224 controls	Cases=GFR< 60	Sex and Age	OR=1.0 (0.4-2.3)
	Callejas Callejas, Alonso Medrano, Mendoza Canales 2003	Cross-Sectional	Communities in Leon and Chinandega	N=997 102 with elevated CR and 895 without elevated CR	Cases: Cr >= 1.5	None	OR=3.9 (2.1-7.4) for agricultural work; OR=1.5 (1.0-2.5) for sugar cane work, OR ranges from 0.9 (gardening) to 4.0 (banana) for other types of cultivation
	Callejas Callejas, Alonso Medrano, Mendoza Canales 2003	Cross-sectional	Non-sugar cane workers in Chinandega	326 workers screened; 24 cases with CR>=1.5	Cases: CR>=1.5; Non cases: Remainder	None	ORs elevated for all occupations: day laborers (OR=2.4), farmers (4.2), and livestock keepers (3.2), shopkeepers (2.1). Elevated risks for all crops but numbers are small.
	Aragon, Torres Lacourte, Gonzalez 2008	Cross-sectional	Five communities in Northeast Leon	N=190 12 with Stage 3-5 (GFR<=60) Prev=6.3%	GRF<=60	None	OR=2.5 (0.8-8.3) for hydrocarbon exposure at work OR=1.9 (0.6-5.7) for cotton work OR=1.1 (0.5-3.2) for agricultural work

**Table 10b Pesticides**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
6	Garcia-Trabanino, Dominguez, Jansa, Oliver 2004	Cross-sectional	Two communities in El Salvador: 1 in coastal area and 1 in high elevation area	291 male volunteers from coastal region; 62 male volunteers from control community	Cases=Proteinuria and CR >1.4	Adjusted for hypertension, diabetes, region, age, agricultural occupation, and alcohol consumption	OR=0.8 (0.4-1.5)
25	Zelaya 2001	Case-Control	Workers from Ingenio San Antonio	468 cases and 468 controls	Cases=Diagnosis of CKD	None in this analysis.	OR=1.9 (1.1-3.3)
26	Castrillo, Bonilla, Estrada 2001	Case-Control	Hospital-based in Leon	65 cases and 130 controls	Cases=CKD symptoms and CR >1.2	Sex and age matching	OR=2.6 (1.1-6.1)
46	Ruguma 2001	Case-control	Hospital-based in Leon	165 cases and 334 controls	Cases=CR>=1.2; with complications Controls=Other internal medicine patients without elevated CR	None in primary analysis; data stratified by sex, urban/rural residence	OR=9.3 (5.4-16.3) overall Rural males OR=10.1 (4.4-23.7) Urban males OR=4.0 (1.2-13.1) Few females
58	Torres, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in Chichigalpa	327 subjects	Stages 3 and 4: GFR <=60 n=159	Stratification by location of residence (rural/urban)	OR=4.3 (p<.01) for current pesticide exposure among urban subjects and OR=14.6 (p<.01) for current exposure among rural subjects
59	Torres Lacourt, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in La Isla and Candelaria	175 in La Isla and 202 in Candelaria (94-95% participation rates; 38-43% of population)	Stages 3 and 4: GFR <=60 n=76 in La Isla and 84 in Candelaria	No	OR=2.1 (1.0-4.5) in La Isla and OR=4.8 (2.3-10.0) in Candelaria
67	Lopez Arteaga 2005	Case-Control	Community-based in Leon and Chinandega	490 cases and 3320 controls	Cases= CR>1.3 and GFR<80; Controls=CR <1.3 and GFR >80	Only male workers included; conducted a logistic regression which controlled for age, family history of CRI and cancer, alcohol consumption, history of kidney infections, use of NSAIDS, diabetes and	OR=2.3 (1.7-3.2)

						hypertension	
	Sister City Health Committee 2008	Case-Control	Community-based in Quezalguaque	95 cases and 224 controls	Cases=GFR< 60	Sex and Age	OR=1.9 (0.9-3.9)
	Sequeira 2003	Cross-sectional	Non-sugar cane farm workers (coffee, corn, bean) in Jinotega	1000 workers tested; 7 cases of CRI found	Case=CR>1.5	None	43% of cases used pesticides; unknown percent among non-cases
	Callejas Callejas, Alonso Medrano, Mendoza 2003	Case-Control	Hospital-based in El Viejo, Chinandega. All sugar cane workers	38 cases 111 controls	Case=CR>=1.5	None	OR=0.6 (for pesticide application)
	Alonso Medrano, Perea 2002	Case-control	Sugar cane workers in Chichigalpa	44 cases 100 controls	Cases: workers since 1999 with symptoms and CR>=2.5; Controls: active workers without symptoms and CR<1.1	None	OR=1.6 (0.8-3.6)
	Callejas Callejas, Alonso Medrano, Mendoza Canales 2003	Cross-Sectional	Communities in Leon and Chinandega	997 subjects 102 with elevated CR 895 without elevated CR	Cases: Cr >= 1.5	None	OR for pesticide application= 2.2 (1.4-3.4) OR for pesticide intoxication=2.2 (1.3-3.7)
	Aragon, Torres Lacourte, Gonzalez 2008	Cross-sectional	Five communities in Northeast Leon	190 subjects 12 with Stage 3-5 (GFR<=60) Prev=6.3%	GRF<=60	None	OR for pesticide application=0.5 (0.1-2.1) OR for pesticide intoxication=1.1 (0.2-8.2)

**Table 10c Heavy Metals**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
40	Marin, Berroteran 2002	Case-Control	Workers from Ingenio San Antonio	43 cases, 39 controls	Cases= workers with CKD, Controls=workers without CKD	Unknown	OR=18.9 for lead. No p value reported.
49	Zelaya Rivas 2001	Case-Control	Workers from Ingenio San Antonio	468 cases and 468 controls; lead was evaluated in blood and hair from 57 cases and 68 controls	Cases=Diagnosis of CKD	None	Numerical data not reported. "Blood lead levels were higher in patients in Leon and Chinandega which could be a manifestation of diminished excretion from kidneys."
49	Unknown (reported by Zelaya) 2000-2001	Case-Control	Farm workers in the western region	600 cases and 600 controls	Cases=CRI; unknown definition	Unknown	"There were high lead levels in hair samples of both cases and controls." Cadmium and arsenic levels in hair also examined but no results were reported.
56-57	Torres, Gonzalez, Aragon, Wesseling, Lundberg 2007	Cross-sectional	Five northwestern communities with working populations: mine workers, agricultural workers; service workers; fishermen; coffee growers	479 women, 617 men	Cases=CR >1.2 for males and CR>0.9 for females	Not yet analyzed	Not yet analyzed
64	Uriarte Barrera, Valerio Vasquez, Zamora Roque 2000	Case-Control	Workers from Ingenio San Antonio	15 cases, 15 controls	Cases=Workers at ISA hospital with CKD diagnosis Controls=Workers at ISA hospital without CKD	Stratified on smoking	Total mean cadmium exposure 0.73 ug/day among all cases vs. 0.42 among all controls (p value= 0.12). Total mean cadmium exposure 0.95 ug/day among smoking cases vs. 0.53 among smoking controls (p value= 0.09). No association was seen among non-smokers.
	Sister City Health Committee 2008	Case-Control	Community-based in Quezalguaque	95 cases and 224 controls	Cases=GFR< 60	Not in the current analysis	Only one of 771 community members was found with high blood lead; he had a normal GFR

**Table 10d Dehydration**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
25	Zelaya 2001	Case-Control	Workers from Ingenio San Antonio	468 cases and 468 controls	Cases=Diagnoses of CKD	Yes but particular variables not well-justified	Crude OR=11.1 for high temperatures Adjusted OR=4.2 for high temperatures
46	Rugama 2001	Case-control	Hospital-based in Leon	165 cases and 334 controls	Cases=CR>=1.2 ; with complications Controls=Other internal medicine patients without elevated CR	None in primary analysis; data stratified by sex, urban/rural residence	OR=4.2 (2.4-7.1) for intensive labor at high temperatures
49	Unknown (reported by Zelaya) 2000-2001	Case-Control	Farm workers in the western region	600 cases and 600 controls	Cases=CRI; unknown definition	Unknown	“Workers who did not get sick consumed at least 10 liters of electrolyte solution compared to 3 liters among those who did get sick.”
58	Torres, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in Chichigalpa	327 subjects	Stages 3 and 4: GFR <=60; n=159	Stratification by location of residence (rural/urban)	OR=0.4(p=.8) among urban subjects and OR=0.7 (p=.6) among rural subjects for extreme thirst at work
59	Torres Lacourt, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in La Isla and Candelaria	175 in La Isla and 202 in Candelaria (94-95% participation rates; 38-43% of population)	Stages 3 and 4: GFR <=60 n=76 in La Isla and 84 in Candelaria	None	OR=1.2 (0.5-2.6) in La Isla and OR=0.2 (0.1-0.6) in Candelaria for thirst
	Sister City Health Committee 2008	Case-Control	Community-based in Quezalguaque	95 cases and 224 controls	Cases=GFR < 60	Sex and Age	OR=1.8 (1.0-3.6) for thirst
	Aragon, Torres Lacourte, Gonzalez 2008	Cross-sectional	Five communities in Northeast Leon	190 subjects N=12 with Stage 3-5 (GFR<=60) Prev=6.3%	GRF<=60	None	OR for thirst during work=3.9(1.1-13.8)

**Table 10e Urinary Tract Infections**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
25	Zelaya 2001	Case-Control	Workers from Ingenio San Antonio	468 cases and 468 controls	Cases=Diagnosis of CKD	None in UTI analysis; Yes for Kanamicina analysis but particular variables not well-justified	UTI crude OR=1.9 (95% CI= 1.4-2.6) Kanamicina use crude OR=2.4; adjusted OR= 2.0 (1.1-3.5)
26	Castrillo, Bonilla, Estrada 2001	Case-Control	Hospital-based in Leon	65 cases and 130 controls	Cases=CKD symptoms and CR >1.2	Sex and age matching	OR=3.2 (1.5-7.2)
58	Torres, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in Chichigalpa	327 subjects	Stages 3 and 4: GFR <=60	Stratification by location of residence (rural/urban)	OR=5.4 (p<.01) among urban subjects and OR=0.6 (p=.5) among rural subjects
59	Torres Lacourt, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in La Isla and Candelaria	175 in La Isla and 202 in Candelaria (94-95% participation rates; 38-43% of population)	Stages 3 and 4: GFR <=60 n=76 in La Isla and 84 in Candelaria	None	OR=2.8 (1.0-7.8) in La Isla and OR=1.7 (0.7-4.3) in Candelaria
67	Lopez Arteaga 2005	Case-Control	Community-based in Leon and Chinandega	490 cases and 3320 controls	Cases= CR>1.3; Controls=CR <1.3	Only male workers included; conducted a logistic regression which controlled for age, family history of CRI and cancer, alcohol consumption, pesticide use, use of NSAIDS, diabetes and hypertension	OR=1.4 (0.9-2.4) for history of kidney infections
	Alonso Medrano, Perea 2002	Case-control	Sugar cane workers in Chichigalpa	44 cases 100 controls	Cases: workers since 1999 with symptoms and CR>=2.5; Controls: active workers without symptoms and CR<1.1	None	Frequent urinary tract infections=18% among cases and 19% among controls
	Callejas Callejas, Alonso Medrano, Mendoza	Case-Control	Hospital-based in El Viejo, Chinandega. All sugar cane	38 cases 111 controls	Case=CR>=1.5	Certain analyses	OR=0.5 for repeated urinary tract infection (crude)

	2003		workers.				
	Callejas Callejas, Alonso Medrano, Mendoza 2003	Cross-Sectional	Communities in Leon and Chinandega	N=997 102 with elevated CR and 895 without elevated CR	Cases: Cr $\geq$ 1.5	None	OR=0.7 (0.4-1.2)
	Callejas Callejas, Alonso Medrano, Mendoza Canales 2003	Cross-sectional	Non-sugar cane workers in Chinandega	326 workers screened; 24 cases with CR $\geq$ 1.5	Cases: CR $\geq$ 1.5; Non cases: Remainder	None	38% of cases and 30% of non-cases have a history of repeated urinary tract infections
	Aragon, Torres Lacourte, Gonzalez 2008	Cross-sectional	Five communities in Northeast Leon	N=190 12 with Stage 3-5 (GFR $\leq$ 60) Prev=6.3%	GRF $\leq$ 60	None	OR =3.8 (0.9-16.9)

**Table 10f Diabetes**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
3	Dominguez, Moya Perez, Maria Jansa 2003	Cross-sectional	Four countries among Pacific Coast: Mexico, Guatemala, El Salvador, Honduras	806 male residents with chronic pesticide exposure	Cases=Proteinuria	None	4% of cases and 7% of non-cases have diabetes
6	Garcia-Trabanino, Dominguez, Jansa, Oliver 2004	Cross-sectional	Two communities in El Salvador: 1 in coastal and 1 in high elevation area	291 male volunteers from coastal region; 62 male volunteers from control community	Cases=Proteinuria and CR >1.4	Hypertension, diabetes, age, region, agricultural work, alcohol use	OR=2.1 (1.2-3.6)
25	Zelaya 2001	Case-Control	Workers from Ingenio San Antonio	468 cases and 468 controls	Cases=Diagnosis of CKD	None	OR=0.7 (0.3-0.8)
26	Castrillo, Bonilla, Estrada 2001	Case-Control	Hospital-based in Leon	65 cases and 130 controls	Cases=CKD symptoms and CR >1.2	Sex and age matching	OR=1.8 (0.8-4.0)
56-57	Torres, Gonzalez, Aragon, Wesseling, Lundberg 2007	Cross-sectional	Five northwestern communities with working populations: mine workers, agricultural workers; service workers; fishermen; coffee growers	479 women and 617 men	Cases=CR >1.2 for males and 0.9 for females	Age, sex, and community	ORs=0.9 (0.1-5.9) among men and 1.2 (0.5-3.0) among women
58	Torres, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in Chichigalpa	327 subjects	Stages 3 and 4: GFR <=60; n=159	Stratification by location of residence (rural/urban)	OR=3.5 (p=.02) among urban subjects; no diabetes among rural subjects
59	Torres Lacourt, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in La Isla and Candelaria	175 in La Isla and 202 in Candelaria (94-95% participation rates; 38-43% of population)	Stages 3 and 4: GFR <=60 n=76 in La Isla and n=84 in Candelaria	None	OR=3.4 (1.1-10.6) in La Isla and OR=1.5 (0.4-5.7) in Candelaria
67	Lopez Arteaga 2005	Case-Control	Community-based in Leon and Chinandega	490 cases and 3320 controls	Cases= CR>1.3; Controls=CR <1.3	Only male workers included; conducted a logistic regression which controlled for age, family	OR=0.7 (0.1-4.6)

						history of CRI and cancer, pesticide use, alcohol consumption, history of kidney infections, use of NSAIDS, and hypertension	
	Sister City Health Committee 2008	Case-Control	Community-based in Quezalguaque	95 cases and 224 controls	Cases=GFR < 60	Sex and Age	OR=0.7 (0.2-2.0)
	Alonso Medrano, Perea 2002	Case-control	Sugar cane workers in Chichigalpa	44 cases 100 controls	Cases: workers since 1999 with symptoms and CR>=2.5; Controls: active workers without symptoms and CR<1.1	None	0% of cases and 2% of controls reported diabetes
	Callejas Callejas, Alonso Medrano, Mendoza 2003	Case-Control	Hospital-based in El Viejo, Chinandega; All sugar cane workers	38 cases 111 controls	Case=CR>=1.5	Certain analyses	No cases or controls reported a history of diabetes
	Callejas Callejas, Alonso Medrano, Mendoza 2003	Cross-Sectional	Communities in Leon and Chinandega	N=997 102 with CR>=1.5 895 with CR <1.5	Cases: Cr >= 1.5	None	OR=1.7 (0.5-5.1)
	Callejas Callejas, Alonso Medrano, Mendoza Canales 2003	Cross-sectional	Non-sugar cane workers in Chinandega	326 workers screened; 24 cases with CR>=1.5	Cases: CR>=1.5; Non cases: Remainder	None	8% of cases and 1% of non-cases
	Alonso, Callejas Callejas, Dominiguez, Moya 2003	Cross-sectional	Male residents of coastal area of Chinandega who are exposed to pesticides and herbicides	N=210	Case=Proteinuria	None	9% of cases and 5% of non cases
	Aragon, Torres Lacourte, Gonzalez 2008	Cross-sectional	Five communities in Northeast Leon	N=190 12 with Stage 3-5 (GFR<=60) Prev=6.3%	GRF<=60	None	Only two subjects had diabetes

**Table 10g Hypertension**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case Definition	Confounders Controlled?	Results
3	Dominguez, Moya Perez, Maria Jansa 2003	Cross-sectional	Four countries among Pacific Coast: Mexico, Guatemala, El Salvador, Honduras	806 male residents with chronic pesticide exposure	Cases=Proteinuria	None	6% of cases and 12% of non-cases have hypertension
6	Garcia-Trabanino, Dominguez, Jansa, Oliver 2004	Cross-sectional	Two communities in El Salvador: 1 in coastal and 1 in high elevation area	291 male volunteers from coastal region; 62 male volunteers from control community	Cases=Proteinuria and CR >1.4	Hypertension, diabetes, age, region, agricultural work, alcohol use	Odds ratio 2.3 (1.2-4.4)
25	Zelaya 2001	Case-Control	Workers from Ingenio San Antonio	468 cases and 468 controls	Cases=Diagnosis of CKD	None	OR= 4.4 (3.0-6.4)
26	Castrillo, Bonilla, Estrada 2001	Case-Control	Hospital-based in Leon	65 cases and 130 controls	Cases=CKD symptoms and CR >1.2	Sex and age matching	OR=6.3 (3.1-12.0)
56-57	Torres, Gonzalez, Aragon, Wesseling, Lundberg 2007	Cross-sectional	Five northwestern communities with working populations: mine workers, agricultural workers; service workers; fishermen; coffee growers	479 women and 617 men	Cases=CR >1.2 for males and 0.9 for females	Age, sex, and community	ORs=2.0 (1.7-3.4) among men and 1.3 (0.8-2.0) among women
58	Torres, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in Chichigalpa	327 subjects	Stages 3 and 4: GFR <=60; n=159	Stratification by location of residence (rural/urban)	OR=1.9 (p=.15) among urban subjects and OR=3.1 (p=.131) among rural subjects
59	Torres Lacourt, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in La Isla and Candelaria	175 in La Isla and 202 in Candelaria (94-95% participation rates; 38-43% of population)	Stages 3 and 4: GFR <=60 n=76 in La Isla and n=84 in Candelaria	No	OR=0.6 (0.3-2.0) in La Isla and OR=1.4 (0.6-3.2) in Candelaria
67	Lopez Arteaga 2005	Case-Control	Community-based in Leon and Chinandega	490 cases and 3320 controls	Cases= CR>1.3; Controls=CR <1.3	Only male workers included; conducted a logistic regression which controlled for age, family	OR=0.7 (0.4-1.1)

						history of CRI and cancer, pesticide use, alcohol consumption, history of kidney infection, use of NSAIDS, and diabetes	
	Sister City Health Committee 2008	Case-Control	Community-based in Quezalguaque	95 cases and 224 controls	Cases=GFR < 60	Sex and Age	OR=1.4 (0.8-2.3)
	Alonso Medrano, Perea 2002	Case-control	Sugar cane workers in Chichigalpa	44 cases 100 controls	Cases: workers since 1999 with symptoms and CR >= 2.5; Controls: active workers without symptoms and CR < 1.1	None	History of hypertension = 10% among cases and 6% among controls
	Callejas Callejas, Alonso Medrano, Mendoza 2003	Cross-Sectional	Communities in Leon and Chinandega	N=997 102 with CR >= 1.5 and 895 with CR < 1.5	Cases: Cr >= 1.5	None	OR=1.7 (0.9-3.1)
	Callejas Callejas, Alonso Medrano, Mendoza 2003	Case-Control	Hospital-based in El Viejo, Chinandega. All sugar cane workers	38 cases 111 controls	Case=CR >= 1.5	Certain analyses	11% of cases and 0% of controls reported a history of hypertension
	Callejas Callejas, Alonso Medrano, Mendoza Canales 2003	Cross-sectional	Non-sugar cane workers in Chinandega	326 workers screened; 24 cases with CR >= 1.5	Cases: CR >= 1.5; Non cases: Remainder	None	13% of cases and 7% of non-cases
	Alonso, Callejas Callejas, Dominiguez, Moya 2003	Cross-sectional	Male residents of coastal area of Chinandega who are exposed to pesticides and herbicides	N=210	Case=Proteinuria	None	18% of cases and 6% of non cases

**Table 10h Non-Steroidal Anti-inflammatory Drugs (NSAIDs)**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
25	Zelaya 2001	Case-Control	Workers from Ingenio San Antonio	468 cases and 468 controls	Cases=Diagnosis of CKD	None	OR=0.7 (0.6-1.0)
46	Rugama 2001	Case-control	Hospital-based in Leon	165 cases and 334 controls	Cases=CR $\geq$ 1.2 with complications Controls=Other internal medicine patients without elevated CR	None in primary analysis; data stratified by sex, urban/rural residence	OR=4.2 (2.4-7.1)
49	Unknown (reported by Zelaya) 2000-2001	Case-Control	Farm workers in the western region	600 cases and 600 controls	Cases=CRI; unknown definition	Unknown	“The diseased consumed self-prescribed nephrotoxic medications (NSAIDs) four-times more frequently than those who did not get sick.”
58	Torres, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in Chichigalpa	327 subjects	Stages 3 and 4: GFR $\leq$ 60; n=159	Some stratification by age, sex, and location of residence (rural/urban)	OR=0.5 (p=.18) among urban subjects; no NSAIDs among rural subjects
59	Torres Lacourt, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in La Isla and Candelaria	175 in La Isla and 202 in Candelaria (94-95% participation rates; 38-43% of population)	Stages 3 and 4: GFR $\leq$ 60 n=76 in La Isla and n=84 in Candelaria	None	OR=0.7 (0.3-1.6) in La Isla and OR=0.5 (0.2-1.2) in Candelaria
67	Lopez Arteaga 2005	Case-Control	Community-based in Leon and Chinandega	490 cases and 3320 controls	Cases= CR $>$ 1.3; Controls=CR $<$ 1.3	Only male workers included; conducted a logistic regression which controlled for age, family history of CRI and cancer, pesticide use, alcohol consumption, history of kidney infections, diabetes and hypertension	OR=1.2 (0.9-1.5)
	Callejas Callejas, Alonso Medrano, Mendoza	Case-Control	Hospital-based in El Viejo, Chinandega. All sugar cane	38 cases 111 controls	Case=CR $\geq$ 1.5	Certain analyses	OR=1.1 (0.5-2.5) (crude)

	2003		workers.				
	Sister City Health Committee 2008	Case-Control	Community-based in Quezalguaque	95 cases and 224 controls	Cases=GFR < 60	Sex and Age	OR=1.1 (0.7-1.9)
	Alonso Medrano, Perea 2002	Case-control	Sugar cane workers in Chichigalpa	44 cases 100 controls	Cases: workers since 1999 with symptoms and CR ≥ 2.5; Controls: active workers without symptoms and CR < 1.1	None	OR = 1.3 (0.6-2.8)
	Aragon, Torres Lacourte, Gonzalez 2008	Cross-sectional	Five communities in Northeast Leon	N=190 12 with Stage 3-5 (GFR ≤ 60) Prev=6.3%	GFR ≤ 60	None	OR=1.2 (0.4-3.6) for analgesics and anti-inflammatory drugs

**Table 10i Lija/ Alcohol**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
3	Dominguez, Moya Perez, Maria Jansa 2003	Cross-sectional	Four countries among Pacific Coast: Mexico, Guatemala, El Salvador, Honduras	806 male residents with chronic pesticide exposure	Cases=Proteinuria	None	56% of cases and 51% of non-cases have history of "alcoholism"
6	Garcia-Trabanino, Dominguez, Jansa, Oliver 2004	Cross-sectional	Two communities in El Salvador: 1 in coastal and 1 in high elevation area	291 male volunteers from coastal region; 62 male volunteers from control community	Cases=Proteinuria and CR >1.4	Hypertension, diabetes, age, region, agricultural work, alcohol use	OR=0.7 (0.4-1.2) for alcohol
25	Zelaya 2001	Case-Control	Workers from Ingenio San Antonio	468 cases and 468 controls	Cases=Diagnosis of CKD	None	OR for lija =5.7 (4.2-7.9) OR for alcohol=1.8 (1.2-2.5)
26	Castrillo, Bonilla, Estrada 2001	Case-Control	Hospital-based in Leon	65 cases and 130 controls	Cases=CKD symptoms and CR >1.2	Sex and age matching	OR for alcohol =2.6 (0.7-9.8)
40	Marin Ruiz, Berroteran 2002	Case-Control	Workers from Ingenio San Antonio	43 cases, 39 controls	Cases= workers with CKD, Controls=workers without CKD	Unknown	OR for alcohol =7.6
49	Unknown (reported by Zelaya) 2000-2001	Case-Control	Farm workers in the western region	600 cases and 600 controls	Cases=CRI; unknown definition	Unknown	"The diseased had two-times greater ingestion of lija."
58	Torres, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in Chichigalpa	327 subjects	Stages 3 and 4: GFR <=60; n=159	Some stratification by age, sex, and location of residence (rural/urban)	OR for alcohol =3.1 (p<.01) among urban subjects and OR=2.6 (p=.2) among rural subjects
59	Torres Lacourt, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in La Isla and Candelaria	175 in La Isla and 202 in Candelaria 94-95% participation rates; 38-43% of population	Stages 3 and 4: GFR <=60 n=76 in La Isla and n=84 in Candelaria	No	OR for alcohol =3.2 (1.5-6.8) in La Isla and OR=1.9 (0.9-4.4) in Candelaria
67	Lopez Arteaga 2005	Case-Control	Community-based in Leon and Chinandega	490 cases and 3320 controls	Cases= CR>1.3; Controls=CR <1.3	Only male workers included; conducted a logistic regression which controlled for age, family history of CRI and cancer, pesticide use, history of	OR for alcohol =1.4 (1.1-1.8)

						kidney infections, use of NSAIDS, diabetes and hypertension	
	Sister City Health Committee 2008	Case-Control	Community-based in Quezalguaque	95 cases and 224 controls	Cases=GFR< 60	Sex and Age	OR=1.7 (0.8-3.5)
	Callejas Callejas, Alonso Medrano, Mendoza 2003	Case-Control	Hospital-based in El Viejo, Chinandega; All sugar cane workers	38 cases 111 controls	Case=CR>=1.5	Certain analyses: age, etc. Justification for variables= unclear.	OR for lija =11.0 (3.8-21.8) (adjusted) OR for alcohol=2.2 (0.7-7.7) (crude)
	Sequeira 2003	Cross-sectional	Non-sugar cane farm workers in Jinotega	1000 workers tested; 7 cases of CRI found	Case=CR>1.5	None	71% of cases consumed alcohol; most non-cases also consumed alcohol
	Alonso Medrano, Perea 2002	Case-control	Sugar cane workers in Chichigalpa	44 cases 100 controls	Cases: workers since 1999 with symptoms and CR>=2.5; Controls: active workers without symptoms and CR<1.1	Multivariate analysis was mentioned for lija. Controlled for age, years in active cane cutting, cabrito and pathological antecedents	Alcohol: Crude OR = 4.3 (1.3-15.8) Crude Lija OR= 10.8 (3.6-29.6) Adjusted Lija OR = 6.7
	Callejas Callejas, Alonso Medrano, Mendoza Canales 2003	Cross-sectional	Non-sugar cane workers in Chinandega	326 workers screened; 24 cases with CR>=1.5	Cases: CR>=1.5; Non cases: Remainder	None	Alcohol consumption: 100% of case and 89% of non cases Lija consumption: OR=4.8 (1.7-13.7)
	Callejas, Alonso, Mendoza 2003	Cross-Sectional	Communities in Leon and Chinandega	N=997 102 with CR>=1.5 895 with CR<1.5	Cases: CR >= 1.5	None	Alcohol: OR = 3.7 (1.9-7.6) Lija: OR = 4.2 (2.6-7.0)
	Alonso, Callejas Callejas, Dominiguez, Moya 2003	Cross-sectional	Male residents of coastal area of Chinandega who are exposed to pesticides and herbicides	N=210	Case=Proteinuria	None	76% of cases and 63% of non cases have "alcoholism"
	Aragon, Torres Lacourte, Gonzalez 2008	Cross-sectional	Five communities in Northeast Leon	N=190 12 with Stage 3-5 (GFR<=60) Prev=6.3%	GRF<=60	None	OR=1.2 (0.3-5.2) for past alcohol consumption

**Table 10j Cigarette Smoking**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
3	Dominguez, Moya Perez, Maria Jansa 2003	Cross-sectional	Four countries among Pacific Coast: Mexico, Guatemala, El Salvador, Honduras	806 male residents with chronic pesticide exposure	Cases=Proteinuria	None	53% of cases and 58% of non-cases have history of "tobacco addiction"
58	Torres, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in Chichigalpa	327 subjects	Stages 3 and 4: GFR <=60; n=159	Some stratification by age, sex, and location of residence (rural/urban)	OR=1.8 (p<.23) among urban subjects and OR=2.0 (p=.5) among rural subjects
59	Torres Lacourt, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in La Isla and Candelaria	175 in La Isla and 202 in Candelaria (94-95% participation rates; 38-43% of population)	Stages 3 and 4: GFR <=60 n=76 in La Isla and n=84 in Candelaria	None	OR=3.8 (1.8-8.0) in La Isla and OR=3.6 (1.7-7.7) in Candelaria
	Alonso, Callejas Callejas, Dominiguez, Moya 2003	Cross-sectional	Male residents of coastal area of Chinandega who are exposed to pesticides and herbicides	N=210	Case=Proteinuria	None	58% of cases and 56% of non cases have tobacco "addiction"
	Sister City Health Committee 2008	Case-Control	Community-based in Quezalguaque	95 cases and 224 controls	Cases=GFR< 60	Sex and Age	OR=1.0
	Aragon, Torres Lacourte, Gonzalez 2008	Cross-sectional	Five communities in Northeast Leon	N=190 12 with Stage 3-5 (GFR<=60) Prev=6.3%	GRF<=60	None	OR=1.0 (0.1-7.2) for past tobacco use OR=0.7 (0.1-5.1) for current use

**Table 10k Family History of Kidney Disease**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
25	Zelaya 2001	Case-Control	Workers from Ingenio San Antonio	468 cases and 468 controls	Cases=Diagnosis of CKD	None	OR for death from renal failure in a family member=1.8
26	Castrillo, Bonilla, Estrada 2001	Case-Control	Hospital-based in Leon	65 cases and 130 controls	CKD symptoms and CR >1.2	Sex and age matching	OR for CKD=4.0 (1.7-9.4)
58	Torres, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in Chichigalpa	327 subjects	Stages 3 and 4: GFR <=60; n=159	Some stratification by age, sex, and location of residence (rural/urban)	OR=0.4 (0.2-3.0) among urban subjects and OR=0.9 (0.1-7.3) among rural subjects
59	Torres Lacourt, Gonzalez, Vanegas, Aragon 2008	Cross-sectional	Community-based in La Isla and Candelaria	175 in La Isla and 202 in Candelaria (94-95% participation rates; 38-43% of population)	Stages 3 and 4: GFR <=60 n=76 in La Isla and n=84 in Candelaria	No	ORs about 1.0 in La Isla and Candelaria
67	Lopez Arteaga 2005	Case-Control	Community-based in Leon and Chinandega	490 cases and 3320 controls	Cases= CR>1.3; Controls=CR <1.3	Only male workers included; conducted a logistic regression which controlled for age, pesticide use, alcohol consumption, history of kidney infections, family history of cancer, use of NSAIDS, diabetes and hypertension	OR for CKD=1.6 (1.1-2.4) for CKD
	Alonso Medrano, Perea 2002	Case-control	Sugar cane workers in Chichigalpa	44 cases 100 controls	Cases: workers since 1999 with symptoms and CR>=2.5; Controls: active workers without symptoms and CR<1.1	None	48% of cases and 29% of controls reported a family history of CRI
	Callejas Callejas, Alonso Medrano, Mendoza 2003	Case-Control	Hospital-based in El Viejo, Chinandega; All sugar cane workers	38 cases 111 controls	Case=CR>=1.5	Certain analyses	13% of cases and 5% of controls reported a family history of chronic renal insufficiency
	Callejas Callejas, Alonso	Cross-Sectional	Communities in Leon and Chinandega	N=997 102 with elevated CR and	Cases: Cr >= 1.5	None	OR=2.0 (1.1-3.9)

	Medrano, Mendoza 2003			895 without elevated CR			
	Callejas Callejas, Alonso Medrano, Mendoza Canales 2003	Cross-sectional	Non-sugar cane workers in Chinandega	326 workers screened; 24 cases with CR>=1.5	Cases: CR>=1.5; Non cases: Remainder	None	13% of cases and 5% of non-cases
	Alonso, Callejas Callejas, Dominiguez, Moya 2003	Cross-sectional	Male residents of coastal area of Chinandega who are exposed to pesticides and herbicides	N=210	Case=Proteinuria	None	50% of cases and 44% of non cases
	Aragon, Torres Lacourte, Gonzalez 2008	Cross-sectional	Five communities in Northeast Leon	N=190 12 with Stage 3-5 (GFR<=60) Prev=6.3%	GRF<=60	None	OR=0.6 (0.1-4.7)

**Table 101 Miscellaneous Exposures**

BU Id No.	Author Year	Type of Study	Study Setting	Number of Subjects	Case and Control Definitions	Confounders Controlled?	Results
46	Ruguma 2001	Case-control	Hospital-based in Leon	165 cases and 334 controls	Cases=CR>=1.2 with complications Controls=other internal medicine patients without elevated creatinine	None in primary analysis; data stratified by sex, urban/rural residence	OR for glucosamine use =3.7 (2.2-6.3)
	Aragon, Torres Lacourte, Gonzalez 2008	Cross-sectional	Five communities in Northeast Leon	N=190 12 with Stage 3-5 (GFR<=60) Prev=6.3%	GRF<=60	None	OR for dengue infection=2.7 (0.8-9.1)

### **III. POTENTIAL CAUSES OF CKD IN NICARAGUA**

#### **A. Introduction**

Based on our review in Section II of the information available on CKD in Nicaragua, while the data provide important clues, it is our view that there is insufficient evidence to draw any conclusions about the cause(s) of the elevated rates of CKD.

Given the elevated rates of CKD in Leon and Chinandega, it is important to note some of the characteristics of these regions, as they affect our choice of the potential hypotheses. One of the most notable geographic features in the region is the presence of a chain of active volcanos which commonly emit ash that reaches the population. In addition to being a respiratory concern, volcanic ash may generally contain heavy metals. Because of the volcanos, the soil is particularly fertile, and the area has always been a prime agricultural region, including not only sugar cane but cotton, peanuts, rice, bananas, and other crops. As a result, there is a long history of pesticide use in the region. Another important environmental feature is the heat. We do not have comparative temperature data, but we have been informed that this region is the hottest and driest of any in Nicaragua. This impression is reinforced by an entry in a tourist guidebook, which states that travelers to Chinandega will know “what it feels like to be a rotisserie chicken” (Wood, 2008). Finally, in the context of raising potential genetic causes, some individuals have noted that the residents of this region are more likely to be of indigenous background. However, we have no information either supporting or contradicting this statement.

In order to appreciate some of the hypotheses that have been included, in particular in relation to the role of heat and strenuous work, it is important to understand some of the developing understanding of CKD. When classifying kidney disease and kidney injury, it is often helpful to dichotomize causes into acute and chronic conditions. Traditional paradigms hold that acute kidney injury (or acute renal failure) is thought to occur in response to an acute event with an abrupt decline in kidney function followed by near total recovery in 2-4 weeks. A typical patient may be one who develops shock due to blood loss during surgery resulting in acute renal failure requiring temporary hemodialysis, but who recovers renal function and returns to their previous baseline kidney function. A minority of patients who suffer acute kidney injury may remain dialysis dependent. In contrast, CKD is thought to develop in response to ongoing damage to the kidney, often from ischemic, immunologic, biochemical, or inflammatory injury. Kidney function worsens slowly over time, and, although the rate of progression may be modified, existing damage is not thought to be reversible. A typical patient may be one with diabetes who, 15 years after developing diabetes, first develops albuminuria, with a subsequent decline in kidney function as measured by an increased serum creatinine, followed by progression to ESRD over the next 10 years.

These paradigms have undergone re-examination in the past few years. It is now recognized that patients suffering acute kidney injury, although apparently recovering to baseline kidney function, may be at increased risk for developing CKD many years in

the future. In fact, it has been shown that patients developing only small changes in serum creatinine during a hospitalization (as small as 0.1 mg/dl) may be at increased risk for developing ESRD (Newsome, 2008; Coca, 2009; Tian, 2009).

As previously discussed, CKD is now not just thought of in terms of etiology, but also in terms of susceptibility factors, initiation factors, and progression factors (Table 4). For example, it has been suggested that infants of low birth weight may have reduced renal mass and nephron number and be more susceptible to CKD later in life. In patients with diabetic nephropathy, it is known that control of blood pressure may be a major factor in preventing progression of CKD, independent of control of blood glucose. In addition, patients with CKD may also be more susceptible to acute kidney injury from such agents as radiographic contrast, nephrotoxic antibiotics, or non-steroidal anti-inflammatory drugs (NSAIDs) and such injury may lead to more rapid progression of the underlying CKD.

Similarly, it is believed that patients who are volume depleted are more likely to develop acute kidney injury when exposed to nephrotoxins, such as radiographic contrast or myoglobin from muscle injury. This hypothesis provides the clinical rationale for treating patients with existing CKD with saline prophylaxis prior to the administration of radiographic contrast to prevent the development of superimposed acute kidney injury (Pannu, 2006). In case series of earthquake victims, the importance of prompt provision of adequate intravenous hydration to victims with crush injuries in order to prevent acute kidney injury from the release of toxic substances from the damaged muscle, is well established (Gunal, 2004).

In summary when considering the development and progression of CKD, it is not just the etiologic agents outlined in Table 3 that are important. Susceptibility factors related to genetics, birth weight, age, prior renal insults, hydration status, and concurrent exposure to nephrotoxins may lead to the development of CKD in some individuals but not others. Once the initial injury is established, such factors as control of blood pressure, smoking, and use of renoprotective or nephrotoxic drugs may change the rate at which the disease becomes clinically manifest. Clearly, in evaluating causality the interplay of multiple factors must be considered (Levey, 2007).

## **B. Specific Hypotheses**

### **1. Agrichemicals**

#### **a. Background**

Agrichemicals, also called pesticides, include herbicides, fungicides and insecticides used to control agricultural “pests” (i.e., plants, fungus, weeds and insects that compete or interfere with agricultural crop production). Agricultural pesticides include a variety of synthetic chemical compounds, often used in combination at different times during production depending on their target pest. The areas of Chinandega and Leon are currently areas of high sugar cane production and historically of high cotton production. Cotton is a high input crop that requires the use of many synthetic chemicals. Sugar cane also requires use of agrichemicals, mostly herbicides known as Atrazine and Roundup. There are concerns among workers that exposure to agrichemicals is a cause of CKD.

#### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

Based on our knowledge of the sex distribution of employment in sugar cane in general in Nicaragua and at ISA in particular, we expect that a substantially greater number of men would be exposed; therefore, the exposure patterns would be consistent with the predominance of CKD among men. Sugarcane is grown around the world in areas where a high prevalence of CKD has not been documented, although it is unclear whether there might be “silent” epidemics.

Nine of thirteen studies that examined pesticide exposure in Nicaragua found modest to strong associations with CKD (Table 10b). Odds ratios ranged from 1.6 to 9.3 and most were statistically significant. However, in these studies exposures to specific pesticides were not examined, and only a few of these studies controlled for confounding variables. The three of the four studies that controlled for at least age and sex found 1.9 to 2.6-fold increased risks (Castrillo, 2001; Lopez Arteaga, 2005; Brookline Sister City Health Committee, 2008). The fourth study conducted in El Salvador was null (Garcia-Trabanino, 2004). The latter included agricultural work as a confounder in the multivariate analysis and so may have masked a possible association with pesticide exposure.

#### **c. Likelihood of causing CKD**

The majority of pesticides used in commercial agricultural today were developed and marketed prior to comprehensive health and safety studies being conducted. Consequently, in many cases we have incomplete data on the possible health effects of such chemicals when used individually, and even less is known of their possible health effects when they are used in combination. Studies on the health effects of individual compounds are usually conducted on laboratory animals, and human risk measures are extrapolated from animal data.

Of the 20 chemicals of concern (described in the following section), Atrazine, Glyphosate (the active ingredient in Roundup and Forza), Paraquat and Nemagon are known to cause kidney damage in animal studies but have not to date been associated with CKD in humans (Bjorge, 1996; EPA, 2009a; EPA, 2009b; EPA, 2009c; Williams, 2000). Paraquat (and diaquat) has been associated with acute renal failure after accidental or intentional toxic exposure (Kim, 2009). However, there are no reports of CKD after long-term exposure, although we are aware of only a single study examining this question which did not find nephrotoxicity after chronic exposure (Senanayake, 1993).

#### **d. Likelihood of exposure**

We have identified 20 pesticides of interest due to the likelihood of exposure. Twelve of these are reportedly used by NSEL in the production of sugar cane. Four additional pesticides are widely used in the sugar cane production in the US as identified in a report prepared by Exponent Inc. (2009), although these four are not reportedly used by NSEL. And finally, four more pesticides have either been used historically or have elsewhere been identified as a potential concern (e.g., Yearout, 2008). See table 11.

Certain jobs at ISA have higher potential for pesticide exposure (e.g., cane cutting vs. factory work), but we do not have enough detail yet to quantify or rank exposure levels.

#### **e. Potential for study**

A number of factors need to be considered regarding pesticide exposures. These include timing of exposure and environmental half-life of the chemical, exposure routes and duration, and amount of exposure. Historic pesticide use may be studied by examining records of purchase and use of pesticides from NSEL. Present or recent exposures may be estimated by measuring soil and water samples for pesticide concentrations, and examining human urine and/or blood for biomarkers of exposure in individuals. However, not all pesticides persist in the environment or in humans and these will be more difficult to measure. This is especially true of Glyphosate and Nemagon.

#### **f. Conclusions/Recommendations**

- i. The main evidence in favor of the agrichemical hypothesis is the highly probable exposure to pesticides among workers.
- ii. The association of pesticides and CKD is unknown. Pesticides exposure is associated with a range of health effects. Kidney damage is a known effect of exposure to some pesticides and it is possible that the effects of more than one type of pesticide are additive or synergistic and that other conditions (e.g., dietary, behavioral) may alter the effect of pesticide exposures.
- iii. We are treating this as a high-priority hypothesis.
- iv. We recommend the development of strategies to sample the environment for concentrations of pesticides in soil and water. This initial strategy will include

input from NSEL and ASOCHIVIDA board members and provide the basis for further investigation.

- v. We recommend observations of practices recommended for safe application of pesticides and protection of workers from exposure.

**Table 11. Pesticides**

<b>Pesticide</b>	<b>NSEL</b>	<b>Exponent</b>	<b>Other</b>
Atrazine (CAS# 1912-24-9)	X	X	
Roundup or Forza (Glyphosate CAS#1071-83-6)	X	X	
Ametrex (Ametryne CAS#834-12-8)	X	X	
Velpar 75 (Hexazinone CAS#51235)	X	X	
Karmex (Diuron CAS#330)	X	X	
Prowl (Pendimethalin CAS# 40487)	X	X	
Finale (Glufosinate ammonium CAS#77182)	X		
Hedonal (2,4-D CAS#94-75-7)	X		
Misil II (Metsulfuron methyl CAS# 7422364-6, Dimethylamine Dicamba CAS#2300-66-5)	X		
Advance (Trifluralin CAS#1582-09-8)	X		
Racumin (Coumatetralyl CAS#5836-29-3)	X		
JADE (Imidacloprid CAS#138261-41-3)	X		
Gramoxeone (Paraquat CAS#1910-42-5)		X	
Terbugran (Terbufos CAS# 13071-79-9)		X	
Coumadin (Warfarin CAS# 81-81-2)		X	
Arrivo (Cypermethrin CAS# 52315-07-8)		X	
Nemagon (dibromochloropropane CAS#96-12-8)			X
DDT (CAS# 50-29-3)			X
DDE (CAS# 72-55-9)			X
DDD (CAS# 72-54-8)			X

## **2. Volume depletion**

### **a. Background**

Although volume depletion is not a recognized cause of CKD, it is a recognized susceptibility factor for acute kidney injury. In fact, the use of prophylactic volume expansion is the cornerstone for the prevention of acute kidney injury due to the administration of nephrotoxic agents such as radiographic contrast or chemotherapeutic drugs (Pannu, 2006). Furthermore several experimental models of nephrotoxic kidney injury require the use of diuretics and/or salt depletion in order for the nephrotoxin to cause kidney injury. These experimental models include aristolochic acid (Debelle, 2002), the likely cause of Balkan endemic nephropathy as well as a potential causative agent of CKD in Nicaragua, and radiocontrast administration (Heyman, 1988).

### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

Available data suggests that CKD prevalence may be higher in occupations in which strenuous work is performed in high environmental temperatures (sugar cane workers, miners). These conditions would predispose to volume depletion. There is further evidence that volume depletion is common in these workers and that adequate rehydration regimens can reduce the risk. An unpublished study found a weight loss of 2.6 kg, an increase in serum sodium to 145, an increase in serum osmolality to 301 mOsm/kg and an increase in urine specific gravity to 1.026 during the work day in a control group, compared to a weight gain of 0.8 kg, an increase in serum sodium to 141, an increase in serum osmolality to 295 mOsm/kg, and an increase in urine specific gravity to 1.015 in a group educated about need for adequate hydration. The results show clear evidence of volume depletion in the control group, while the group receiving the hydration education maintained adequate hydration during the workday (Solis Zepeda, 2007).

### **c. Likelihood of causing CKD**

Unlikely as a primary cause, but may well be a predisposing condition/susceptibility factor, increasing the risk of developing acute kidney injury in the presence of other risk factors. It may also be a progression factor.

### **d. Likelihood of exposure**

Volume depletion has been demonstrated to occur at high environmental temperatures and strenuous labor, consistent with conditions present for many laborers in Nicaragua.

### **e. Potential for study**

Information on volume depletion is probably quite limited from a retrospective review of records. Similarly, interviewing workers to ascertain their hydration habits is likely to be biased. The most direct way to ascertain the importance of this factor is a prospective work observation study.

### **f. Conclusions/Recommendations**

- i. Volume depletion is unlikely the primary cause of CKD, but it is a common and

well-accepted susceptibility factor for kidney injury caused by other agents, particularly due to nephrotoxin exposure. Because it may occur commonly in the population of workers exposed to high environmental temperatures, it is considered to be an important factor in the progression of CKD in Nicaragua.

ii. A prospective work observation study is proposed.

iii. Key data to be collected would be weight immediately before and after work as a measure of fluid loss, as well as orthostatic measurements of blood pressure and pulse as overt signs of volume depletion. Biochemical measures could include pre- and post-creatinine and urine specific gravity or osmolality, but their inclusion may depend on the financial resources available for this specific study.

### **3. Muscle damage**

#### **a. Background**

Muscle damage (rhabdomyolysis) is a well-recognized cause of acute renal failure. Acute renal failure is thought to occur because of the release of the nephrotoxic muscle protein, myoglobin, from damaged muscle. Myoglobinuric acute renal failure has been reported after traumatic muscle damage, such as occurs with crush injury, and after non-traumatic muscle injury, such as occurs in some individuals given statins. It is not typically considered to be a cause of CKD. However, there are isolated reports of chronic interstitial nephritis as a consequence of rhabdomyolysis. Kew et al reported on 40 South African miners who developed heatstroke, all of whom also developed evidence for kidney damage during the acute heatstroke episode (Kew, 1970). Although all the patients who survived the initial episode made complete clinical recoveries, four of the patients went on to develop chronic progressive tubulointerstitial nephritis over a period of four years. There have been no subsequent reports of similar cases, but there has been a report of a patient with McCardle's disease who developed chronic tubulointerstitial nephritis (McCarron, 1980). McCardle's disease is a familial muscle enzyme deficiency characterized by recurrent episodes of rhabdomyolysis and myoglobinuria after exercise, rarely resulting in myoglobinuric acute kidney injury. The reported case had multiple episodes of myoglobinuria with a single episode of acute renal failure at age 42. A kidney biopsy done one month after the episode of acute renal failure, when his serum creatinine was 1.2 mg/dl, showed marked chronic tubulointerstitial disease, which the authors attributed to recurrent episodes of myoglobinuria.

These two reports are important since they provide some histologic evidence for the development of chronic renal injury in response to myoglobinuria. The report of Kew et al is particularly important, since the renal disease occurred in workers under similar environmental conditions to those seen in Nicaragua, specifically strenuous work in high environmental temperatures. However, this report only describes patients with heatstroke, the most severe manifestation of heat induced injury. The report of the

McCardle's patient suggests that chronic myoglobinuria may also be a cause of CKD.

It has been demonstrated that strenuous exercise may result in muscle damage in normal individuals. Clarkson et al studied 203 volunteers aged 18-40 years (Clarkson, 2006). They performed two sets of 25 maximal eccentric contractions of the elbow flexor muscles separated by a five-minute rest. Subjects maximally contracted their elbow flexor muscles to resist the downward movement of a lever moved by the test administrator. All subjects were instructed to drink water during the test. Serum creatine kinase (CK) and myoglobin were measured as markers of muscle damage. Average peak CK levels were 6420, 2100, and 311% and average myoglobin levels were 1137, 170, and 28% above baseline values on days 4, 7 and 10 after exercise. Of these participants, 111 had CK values at 4 days postexercise >2000 U/L and 51 had values >10,000 U/L, levels consistent with the diagnosis of myositis and rhabdomyolysis, respectively. No patient had an alteration in kidney function as measured by creatinine and blood urea nitrogen. Although, not providing evidence for chronic kidney injury, this study does suggest that strenuous exercise may not uncommonly results in significant muscle damage.

The relevance of this report for sugar cane workers in Nicaragua is uncertain. It would seem that if recurrent myoglobinuria caused by strenuous exercise was a cause of chronic kidney disease, that there would be other reports in other workers or endurance athletes. Such reports are lacking. However, it may be a combination of strenuous exercise and volume depletion, potentially in the setting of other initiation factors, which results in CKD in Nicaragua.

**b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

The higher prevalence of CKD in workers in strenuous occupations, such as cane workers and miners, would be consistent with this hypothesis.

**c. Likelihood of causing CKD**

Only limited evidence as a cause of CKD.

**d. Likelihood of exposure**

It is uncertain whether strenuous work in a well-conditioned worker would result in rhabdomyolysis and myoglobinuria.

**e. Potential for study**

Information on rhabdomyolysis is probably quite limited from a retrospective review of records. The most direct way to ascertain the importance of this factor is a prospective work observation study.

**f. Conclusions/Recommendations**

- i. Strenuous exercise with resulting rhabdomyolysis is a well-known cause of acute kidney injury. There is extremely limited information that recurrent myoglobinuria may also result in CKD. With increasing recognition that episodes

of subclinical renal injury may lead to CKD and the likelihood that myoglobinuria may be occurring in cane workers, this etiology deserves specific attention.

ii. A prospective work observation study is proposed.

iii. Key data to be collected would be measurements of serum creatine kinase and myoglobin and urine myoglobin before and after work.

## **4. Systemic Infections**

### **a. Background**

Infectious diseases are an additional possible cause of CKD in Nicaragua, as many infections are associated with both environment (reflecting poor sanitation and hygiene conditions) and occupational exposures. A relationship between occupational exposures, including activities related to sugar cane workers, and an increased risk of infection with diseases, including leptospirosis, hantavirus, malaria, Chagas disease, yellow fever and schistosomiasis, has been described elsewhere (Everard, 1992; David, 2000; Phoolchund J, 1991; Robins, 1998; Rossi, 2007).

### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

This occupational exposure would be consistent with the apparent excess prevalence of CKD in males in the context of Northwestern Nicaragua.

Most of these infectious diseases, when accompanied by acute kidney injury, manifest with an acute and florid symptomatology that would not pass unnoticed. Nevertheless, high seroprevalence of symptomatic and asymptomatic leptospirosis has been described in Nicaragua (David, 2000)

### **c. Likelihood of causing CKD**

Leptospirosis (also known as canefield fever and, in severe cases, referred to Weil's syndrome) can cause acute kidney injury; the lesion is typically consistent with a tubulointerstitial process and may also manifest with electrolyte abnormalities (potentially due to inhibition of the  $\text{Na}^+$ ,  $\text{K}^+$ ,  $2\text{Cl}^-$  transporter in the ascending limb of the Loop of Henle (Wu MS, 2004). Leptospirosis and other endemic infectious diseases may have similar manifestations, although conjunctival suffusion is nearly pathognomonic of leptospirosis.

Other infectious diseases that may have similar features and that are found in Nicaragua include malaria, dengue fever, and hantavirus. Of these, malaria and dengue fever seldom have kidney findings except in the cases of severe illness. Several hantavirus species are known to cause hemorrhagic fever with renal syndrome (HFRS), one form of which is referred to as Nephropathia epidemica (NE). Kidney involvement is more common with hantavirus infection in Europe and Asia and less common in endemic hantavirus in the Western hemisphere. Usually the kidney disease

presents with an acute tubulointerstitial nephritis. Other common interstitial changes include congestion and dilatation of the medullary vessels, hemorrhage into the medullary tissues, interstitial edema, and tubular cell necrosis and degeneration (Mustonen, 1994). Histologic changes in the glomeruli are typically mild despite prominent proteinuria. As with most acute kidney disease, progression to CKD has been noted (Novo, 1999). Antibodies to the virus are detected in the serum, and virus can be demonstrated by PCR in kidney biopsy material. (Papadimitriou M, 1995; Muranyi, 2005).

Parasites can cause secondary glomerulonephritis, probably related to chronic antigen exposure. Parasitic infections endemic to Nicaragua include filariasis, leishmaniasis, and schistosomiasis. Of these, filariasis does not typically have kidney findings although chyluria can occur with severe lymphatic obstruction and both hematuria and proteinuria have been reported (Dreyer G, 1992). Leishmaniasis (particularly cutaneous Leishmaniasis which is more common in Nicaragua) does not typically have kidney findings; and schistosomiasis, when associated with kidney disease, typically manifests with proteinuria.

#### **d. Likelihood of exposure**

Leptospirosis is a zoonosis that can occur through direct or indirect transmission from a mammalian host (rodents, dogs, etc). Indirect transmission via contact with *Leptospira* contaminated water or soil, is thought to be responsible for most cases. For this reason, workers in rice fields, sugar cane plantations and mines (Cespedes Z, 2003) have been described as risk groups. In Western Nicaragua, outbreaks following heavy rains resulting in flooding areas have been described (David, 2000).

Rodents are the principal reservoir for hantavirus. Humans appear to be infected by aerosols or dust from rodent urine, droppings, or by direct contact with saliva through cuts or mucous membranes. People who come in direct contact with rodents and heavily rodent-contaminated areas are at risk, especially rural area residents and farmers. (CDC disease fact sheet).

Both environmental and occupational exposures to these infectious agents are likely to happen in impoverished rural areas of northwestern Nicaragua.

#### **e. Potential for study**

One possible means to study the association between CKD and leptospirosis is to perform serologic testing of previously collected sera from cases and controls. The IgG antibody is a 'long term' marker for past disease, which disappears over time but can remain detectable years after an infection (Cumberland, C.O.R., 2001). If cases had much higher rates of positive Leptospirosis IgG antibody than controls, it would provide support for this infection as a cause of CKD. Unfortunately, there are no specific histologic features of CKD related to Leptospirosis, so a definitive diagnosis cannot be made.

In prospective studies, we recommend inclusion of questions regarding household sanitation systems, water and food sources, ownership of animals, and an estimate of rats or rodents in household.

#### **f. Conclusions/Recommendations**

- i. Infectious diseases such as leptospirosis, hantavirus or malaria are known to cause acute renal failure. There is limited evidence related to their role as causative agents of CKD. However, infectious disease processes may work as precursors or synergistically with other nephrotoxic insults.
- ii. If access to stored samples is available, we will study the presence of leptospirosis IgG antibodies in already collected sera and compare the seroprevalence of cases and controls.

### **Heavy Metals**

Chronic exposure to heavy metals, most notably lead and cadmium with others including mercury, chromium and uranium, is associated with chronic tubulointerstitial nephritis. These heavy metals may accumulate in proximal tubule cells, causing both functional and structural damage that results in reabsorptive and secretory defects. The mechanisms remain unknown but may involve local oxidative stress with associated lipid peroxidation, apoptosis, and necrosis as common phenomena in the course of nephrotoxicity of these metals (Sabolic I, 2006). Little epidemiologic data exist for widespread mercury, chromium and uranium-associated nephropathies aside from sporadic outbreaks generally associated with industrial exposures.

## **5. Lead**

### **a. Background**

The classic heavy metal nephropathy is lead nephropathy, whereby filtered lead accumulates in the proximal tubule, likely resulting in direct tubulotoxicity and subsequent interstitial fibrosis. Resultant hypertension as well as hyperuricemia may serve as progression factors. Lead nephropathy has unique pathologic findings, including acid-fast intranuclear inclusions of proximal tubule cells; in chronic nephropathy, focal tubular atrophy, interstitial fibrosis, and minimal cellular infiltrates predominate. Clinical and laboratory features include decreased urate excretion, proximal tubular dysfunction, and hyporeninemic hypoaldosteronism, with late manifestations including progressive GFR decline, hypertension, and recurrent episodes of (saturnine) gout. Lead nephropathy is difficult to diagnose, as serum levels are unhelpful, typically reflecting bone turnover rather than actual exposure. Accordingly, diagnosis of lead nephropathy is dependent on recognition of patients with an appropriate lead exposure history, CKD, hypertension, and gout.

Most evidence supporting a causal role for lead in CKD comes from studies of occupationally exposed individuals who experienced high levels of exposure (e.g., Weeden, 1975). However, there is also evidence that lower exposure levels stemming from either occupational or environmental sources also have an adverse impact on renal function and may accelerate age-related impairment of renal function (Kim, 1996).

Much of the research data on lead nephropathy and its treatment originates from Taiwan. In one study, following the determination of body lead burden by 72-hour urinary lead excretion after the intravenous infusion of 1 g of calcium disodium EDTA measured by electrothermal atomic-absorption spectrometry, individuals with moderately elevated body lead burden (80 µg to 600 µg) randomized to three months of chelation therapy had significantly lower rates of progression than those not receiving chelation therapy (Lin JL, 2003).

#### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

Epidemiological data: Four epidemiological studies have examined the relationship between lead and CKD in Nicaragua. A case-control study of 43 sick and 39 healthy ISA workers found that 28% of the sick workers and 2.5% of the healthy workers had a positive lead test (Odds Ratio: 18.9) (Lead Presentation). In contrast, a case-control study of lead in hair samples from 600 cases and 600 controls observed similarly high lead levels among both cases and controls (Zelaya FA). The Brookline Sister City Study found only one of 771 community members with high blood lead levels; this individual had a normal GFR (Brookline, 2008). Another case-control study of 57 cases and 68 controls found high blood lead levels among patients in Leon and Chinandega but no results were given comparing cases and controls (Zelaya FA). Lastly, a fifth study of working communities has collected but has not yet analyzed its lead data (Torres, 2007; Torres, 2008).

Clinical pattern: In order to be consistent with the clinical pattern of CKD, lead exposure would need to be more common among men, greater in Chinandega and Leon compared to other areas of the country, and associated with lower socioeconomic status. We currently do not have enough information about distribution of lead exposure to make this assessment.

#### **c. Likelihood of causing CKD**

Based on studies of occupationally exposed individuals, it is possible for even moderate lead exposure over a long duration to adversely impact kidney function. The impact of low levels is less certain. As with prior exposures, lead toxicity may be synergistic with volume depletion and other nephrotoxins.

#### **d. Likelihood of exposure**

Sources of lead exposure in developing countries in South and Central America include gasoline emissions (although this source has been phased-out in many countries), leaded paint, and lead in canned food and beverages. Occupational sources in these areas include lead smelting, mining, and a variety of cottage industries including the

production of lead-glazed ceramics, lead-acid battery processing, and radiator repair work (e.g., Dykeman, 2002; Romieu, 1994). In fact, a community-initiated study in Managua found elevated levels among children living near a battery factory (Morales and Mauss, 1998). (The factory is now closed.) Another possible source of lead in the Nicaraguan environment is volcanic eruptions. In a Dominican population residing in Rhode Island, lead exposure was linked to use of a botanical, litargirio, which is used in the manufacture of batteries, glass, and ceramics; in the vulcanizing of rubber; as a paint pigment; and as an antiperspirant as well as a traditional remedy for burns and fungal infections of the feet (MMWR, 2003). We are unaware of medicinal use of this agent in Nicaragua. Notably, lead exposure, unless linked to occupation or recreational activities (i.e., moonshine consumption in the US after using car radiators as distilleries), would not be more common in men.

It is currently unknown whether there are significant sources of environmental and occupational lead exposure in the affected communities. Low lead levels were observed in nearly all available water samples (75). Only two samples had moderate levels – about 17 ug/L. Levels reported in another data source (47) seem too high (reported as 50-55%) to be accurate. The representativeness of these water samples is unknown. Other environmental media such as soil may also be contaminated.

#### **e. Potential for study**

Environmental sampling of water and soil will help determine the presence of lead as a possible factor in the etiology of CKD. It is also important to determine possible occupational sources.

Lead exposure using biological samples would be difficult to study. To accurately assess lead levels requires either blood levels at the time of exposure (and not at the time of diagnosis of CKD) or assessment of total body lead burden as described above. This testing could theoretically be performed in a subset of individuals but would be labor intensive and not inexpensive. However, as there is available therapy for lead nephropathy which is far less expensive than kidney replacement therapy, it may be worthwhile to more fully investigate total body lead burden in a subpopulation of individuals with stage 3-4 CKD.

#### **Potential study could**

- i. Assess the work and living environment for potential exposures to lead
- ii. If evidence is suggestive, a study of total body lead burden in a subset of individuals with CKD could be undertaken, particularly if environmental sampling identifies sources of lead exposure.

#### **f. Conclusions/Recommendations**

- i. We do not know much about the sources, distribution, and levels of lead exposure in Nicaragua. It would be useful to know the lead levels in water and soil that are accessible to people.

- ii. The epidemiological study results do not present a cohesive picture. Some results are positive; some are null; and some are missing. Reverse causation may explain the positive results.
- iii. Lead levels would have to be very high in order to be the main cause of CKD. However, it is quite possible that lower-level lead exposure is one of many contributing causes. It is also possible that the combined effects of lead and cadmium (the two exposures may co-occur) are synergistic. If so, the source would likely be occupational in nature in order to explain the clinical pattern of CKD in Nicaragua.

## **6. Cadmium**

### **a. Background**

Cadmium is a heavy metal with well-established nephrotoxicity (U.S. Department of Health and Human Services, 2008), often reflecting prolonged low-level exposure (Gonick HC, 2008). Data from human studies, which primarily examine occupational exposure, suggest a latency period of approximately 10 years before clinical onset of renal damage, depending on the intensity of the exposure. However, subtle alterations in renal function have been described after acute exposure in animals. Damage is both tubular and glomerular, although tubular proteinuria appears more prominent than glomerular proteinuria.

Cadmium toxicity may be associated with non-renal manifestations, including crippling and painful osteomalacia with high level exposures. Other renal manifestations may include a Fanconi syndrome (proximal tubular wasting) as well as an immune-complex mediated glomerulonephritis. Chronic cadmium exposure is associated with progressive renal tubular dysfunction in humans, and the toxic effects on the kidney appear to be dose-related. Even very low levels of cadmium exposure may have adverse effects on the kidney, although the lowest dose that induces renal damage is currently unknown.

Occupational exposure to cadmium exposure occurs during mining, work with cadmium containing ores, manufacturing of cadmium containing products including paints and batteries, plating, soldering and welding. These occupational activities contaminate the ambient environment (e.g., air, soil, water) leading to general population exposure through the ingestion of cadmium-contaminated foods and cigarette smoking. In fact, populations living in areas with high cadmium levels in the soil can have high dietary exposures stemming from crop uptake. In Japan, eating contaminated rice has led to "Itai-Itai" disease, a serious kidney and bone disease occurring mainly in women. Except for volcanic eruptions, the sources of cadmium exposure in Nicaragua are unclear.

### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

To date, only one small case-control study has examined cadmium exposure in relation to CKD (Uriarte Barrera ED). Estimated levels were higher among 15 cases versus 15 controls (0.73 ug/day vs. 0.42 ug/day) but the difference appears to be related to differences in cigarette smoking. Another case-control study has reportedly collected hair samples for heavy metal analyses but no cadmium results were observed in available documents (Zelaya FA).

### **c. Likelihood of causing CKD**

Based on studies of occupationally exposed individuals, it is possible for high, moderate, and even low cadmium levels to adversely impact renal function.

### **d. Likelihood of Exposure**

It is currently unknown whether there are significant sources of environmental and occupational cadmium exposure in the affected communities. Low cadmium levels were observed in all available water samples (75); however, the representativeness of these samples is unknown. Other environmental media such as soil may also be contaminated.

### **e. Potential for study**

Environmental sampling of water and soil will help determine the importance of this exposure in the etiology of CKD. It is also important to determine possible occupational sources.

### **f. Conclusions/Recommendations**

- i. We do not know much about the sources, distribution, and levels of cadmium exposure in Nicaragua. It would be useful to know the levels in water and soil that are accessible to people.
- ii. Epidemiological study results are also lacking.
- iii. High cadmium levels could account for the elevated rates of CKD. In addition, it is possible that moderate and even low-level exposure is one of many contributing causes. It is also possible that the combined effects of lead and cadmium (the two exposures may co-occur) are synergistic. If so, the source would likely be occupational in nature in order to explain the clinical pattern of CKD in Nicaragua.

## **7. Uranium**

### **a. Background**

Uranium, the heaviest of the naturally occurring elements, is a metal whose biological effects were described in the literature as early as the 1820s. Animal studies, as well as studies of occupationally exposed persons, have shown that the major health effect of uranium is chemical kidney toxicity, rather than a radiation hazard (Wrenn ME, 1985; Leggett RW, 1989; Taylor DM, 1997; WHO, 1998). Both functional and histologic damage to the proximal tubules has been demonstrated (Haley DP, 1982; Haley DP,

1982; Diamond GL, 1989; Gilman AP, 1998; Gilman AP, 1998). There is no evidence in animal or human studies of uranium toxicity to glomeruli.

Elevated levels have been found in uranium mining as well as in non-uranium-producing communities. In the latter case, the uranium has been introduced into drinking water, not through human activity, but through contact with naturally occurring deposits of uranium minerals.

Two small studies with 50–100 study persons have been published on the kidney toxicity of natural uranium from drinking water. They have shown an association of uranium exposure with increased urinary glucose, alkaline phosphatase, and  $\beta$ -2-microglobulin excretion (Zamora ML, 1998), as well as increased urinary albumin levels (Mao Y, 1995). A third study of the effects of chronic uranium exposure in drinking water found that it is weakly associated with altered proximal tubulus function without a clear threshold, which suggests that even low uranium concentrations in drinking water can cause nephrotoxic effects (P Kurttio, 2002).

Uranium can be distributed throughout the environment by way of human and natural activity, such as volcanoes, wind, and streams. It can be found as dust in the air, and this dust can dissolve in water and settle on plants, and can be found in larger particles in soil (US Department of Health and Human Services, 1999).

**b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

Exposure to uranium could be consistent with excess CKD in Leon and Chinandega, due to the presence of several active volcanos in the area. It would be consistent with an excess of CKD among males only if the primary exposure was occupational. No studies have been conducted in Nicaragua regarding a potential association between uranium exposure and CKD.

**c. Likelihood of Causing CKD**

Based on studies of occupationally exposed individuals, it is possible for high, moderate, and even low uranium levels to adversely impact renal function.

**d. Likelihood of Exposure**

Exposure to uranium is plausible due to the presence of several active volcanos in the region.

**e. Potential for Study**

Uranium can be easily measured in different media, including water and soil.

**f. Conclusions/Recommendations**

- i. High uranium levels could contribute to the elevated rates of CKD.
- ii. We do not know much about the sources, distribution, and levels of uranium exposure in Nicaragua. It would be useful to know the levels in water and soil.

iii. Epidemiological study results are lacking.

## **8. Aristolochic Acid**

### **a. Background**

Aristolochic acid obtained from seeds of the common plant, *Aristolochia*, is a well-known nephrotoxin and has been incriminated as the source of several epidemics of CKD (DeBelle, 2008). It was originally identified as a nephrotoxin after horses that consumed hay contaminated with the seeds of *Aristolochia clematitis* developed tubulointerstitial kidney disease. Subsequently, an epidemic of chronic tubulointerstitial kidney disease was reported from Belgium in women who had taken an herbal medicine, containing aristolochic acid, as part of a weight loss regimen. Besides the development of ESRD, cases were notable for the development of urothelial carcinomas. Numerous cases of chronic interstitial nephritis have been reported from around the world in patients ingesting herbal remedies and the risk of kidney disease and urothelial tumors is increased in Chinese herbalists. These observations, lead to a re-examination of the possible causes of Balkan endemic nephropathy (BEN), a form of chronic interstitial nephritis and urothelial carcinoma affecting people in a specific geographic distribution along the Danube River. Although the disease had been identified in the 1950's, its cause remained unexplained, until the report of the Belgian epidemic. The clinical similarities, including the development of urothelial cancer, stimulated an examination of aristolochic acid as a potential cause of BEN. Aristolochic acid induced DNA adducts were identified in patients with BEN. It was suspected that the source of the nephrotoxin was chronic dietary intake of bread made from wheat contaminated with the seeds of *Aristolochia clematidis*.

### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

The most compelling reasons to investigate aristolochic acid as a potential cause of CKD in Nicaragua are its association with Balkan endemic nephropathy, a similar geographic epidemic of CKD, as well as the ubiquitous distribution of *Aristolochia* species. However, disease caused by this nephrotoxin should not have a male predominance and it is unknown whether there is an increased risk of urothelial carcinoma in the Nicaraguan population.

### **c. Likelihood of causing CKD**

It is a well-established cause of CKD.

### **d. Likelihood of exposure**

In Nicaragua there are 13 species of *Aristolochia* (*Aristolochia anguicida*, *A. constricta*, *A. cruenta*\*, *A. elegans*, *A. grandiflora*, *A. inflata*\*, *A. maxima*, *A. odoratissima*, *A. pilosa*, *A. ringens*\*, *A. stevensii*, *A. tonduzii*, and *A. trilobata*). It is plausible that there could be contamination with flour, as has been hypothesized to occur in BEN. In addition, if herbal remedies are commonly used, these may also be a source of intoxication.

The most widely used species for medicinal purposes (e.g., snakebite, febrifuge, stomachic) in Eastern Nicaragua are *Aristolochia constricta* and *A. trilobata*. In Western Nicaragua the most widely used species for medicinal purposes are *Aristolochia anguicida* (stomachache), *A. constricta* (snakebites), *A. grandiflora* (dysentery, snake and scorpion bites, rheumatism, venereal disease, the roots of this species are very toxic), and *A. odoratissima* (snakebites, stimulant, diuretic, stomachic, and febrifuge). The common names for the species used in Western Nicaragua are raíz de estrella (*A. constricta*) and guaco (*A. grandiflora*, *A. odoratissima*). Most *Aristolochia* species contain Aristolochic Acid known to be both mutagenic and carcinogenic to animals (personal communication with Felix Coe, PhD, University of Connecticut, July 13, 2009).

#### **e. Potential for study**

Direct incrimination as etiologic agent in the population is not possible, since it would require identification of DNA-adducts in renal tissue. Identification of *Aristolochia* species in areas where grain is grown would provide a possible link with food contamination. Further study would depend on reliable assays of aristolochic acid in foodstuffs and herbal remedies.

#### **f. Conclusions/Recommendations**

- i. Because of its established nature as a nephrotoxin, aristolochic acid should be investigated.
- ii. Investigation should identify the plant in fields where foodstuffs are grown and determine if herbal remedies are used and their sources.
- iii. If it is identified as a potential contaminant, flour and other foodstuffs, could be tested for the presence of aristolochic acid.
- iv. If data are available on the occurrence of urothelial cancers in patients with CKD, they may provide further corroboration of aristolochic acid as an etiologic agent.
- v. Depending on the results of the above investigations, it may be worthwhile to examine available renal tissue for the presence of aristolochic acid-induced DNA adducts.

## **9. Medications**

### **a. Background**

Medications are a common cause of acute kidney injury and may be associated with CKD. Medications commonly associated with acute kidney injury are listed in Table 12. One of the classic epidemics of CKD was associated with combination analgesic use, specifically those that contain phenacetin (an acetaminophen precursor). (Sandler DP, 1989) Analgesic nephropathy associated with phenacetin manifested with renal papillary necrosis and chronic interstitial nephritis in the setting of prolonged and excessive consumption. Most compelling regarding an association between analgesic use and CKD is that there has been a marked reduction in CKD due to analgesics following public health interventions that decreased availability of phenacetin-containing analgesic mixtures and other combined analgesics.

**Table 12. Medications associated with acute kidney injury and CKD.  
Medications in bold are those that may be commonly used in Nicaragua.**

Clinical Syndrome		Causative Agent
Acute Kidney Injury	Pre-renal/Hemodynamic	Calcineurin inhibitors Iodinated radiocontrast <b>ACE inhibitors</b> <b>Angiotensin receptor blockers</b> <b>NSAIDS</b>
	Acute Tubular Necrosis	<b>Aminoglycosides</b> Amphotericin B Cisplatin Other antibiotics
	Acute Interstitial Nephritis	<b>Penicillins, cephalosporins</b> Sulfonamides Rifampin <b>NSAIDS</b>
	Post-Renal	Acyclovir Indinivir Sulfadiazide
	Other	Phosphate Cathartic Agents Maltose Containing Preparations
Chronic Kidney Disease	Tubulointerstitial	Lithium <b>Analgesics (phenacitin)</b> Calcineurin inhibitors Cisplatin Nitrosurea
	Glomerular/Nephrotic	Gold <b>NSAIDS</b> Penicillamine

Common non-steroidal anti-inflammatory drugs (NSAIDs) include ibuprofen, naprosyn and diclofenac, all of which are used widely in Nicaragua. Kidney failure associated exclusively with NSAIDs is unusual; rather NSAIDs are more often a cause of acute renal failure in the setting of severe volume depletion or other nephrotoxins as they affect the ability of the renal vasculature to upregulate perfusion in settings of stress. This may predispose renal tubular cells to hypoxic damage. The combination of NSAIDS with ACE inhibitors, intravenous contrast, volume depletion or aminoglycosides is a relatively common cause of acute renal failure, particularly in individuals with either pre-existing kidney disease or with acute systemic illness.

Aminoglycosides are broad-spectrum antibiotics requiring either intravenous or intramuscular administration. They are highly effective against most Gram-negative bacteria and have varying activity against many Gram-positive bacteria. Aminoglycosides are well known to cause kidney failure, with risk factors include pre-

existing kidney disease, concomitant nephrotoxic medications, advanced age, and dehydration/volume depletion. Older studies in the US demonstrate a risk of nephrotoxicity in the 10-20% range (defined by a 50% or greater fall in calculated creatinine clearance) (Moore RD, 1984). Kanamycin remains in use in Nicaragua as it may be given intramuscularly; it is widely available in local pharmacies. Gentamicin is also used.

In addition to conventional prescription medications, traditional or herbal medications may also be a cause of kidney damage. We previously summarized the link between aristolochic acid, a common component of traditional medicines, and cases of CKD in patients taking herbal remedies, as well as the association of ingestion of litargirio and CKD. Besides these established causes of CKD it is possible that other herbal medications may be associated with CKD.

#### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

In communications both with physicians as well as with affected workers, use of analgesics and antibiotics were common. In combination with severe volume depletion, it is possible that acute renal failure could occur. Theoretically, recurrent insults could lead to renal fibrosis and progression to kidney failure. Similarly, there are reports of widespread use of traditional remedies in Nicaragua and these reports are consistent with the use of traditional remedies in societies without access to conventional prescription medication.

#### **c. Likelihood of causing CKD**

Medications are unlikely as a single primary cause in otherwise healthy individuals, but, in the presence of other initiation or susceptibility factors, NSAIDs, other analgesics and aminoglycoside antibiotics may cause acute kidney injury. Similarly, herbal remedies, particularly those containing aristolochic acid, are known causes of CKD.

#### **d. Likelihood of exposure**

Use of these medications is relatively common among agricultural workers in Nicaragua.

#### **e. Potential for study**

Several opportunities to more fully evaluate the role of NSAID, other analgesic, and aminoglycoside use are available:

- i. Conduct qualitative interviews with physicians regarding prescription practices.
- ii. Include questions about these medications in any survey instrument. Aminoglycosides should be subject to reasonable recollection as they would be injected in the setting of a suspected bacterial illness.
- iii. Medical records at the ISA may contain information about medication administration

#### **f. Conclusions/Recommendations**

- i. Because of their common use and established nature as nephrotoxins, NSAID, combination analgesic and aminoglycoside use, as well as the use of traditional

herbal remedies, should be investigated.

- ii. Potential means of investigation include qualitative interviews, subject questionnaires and chart review
- iii. Interviews with key informants (e.g., Nicaraguan experts, traditional practitioners) may provide information about types of herbal medications frequently used in northwestern Nicaragua.

## **10. Alcohol**

### **a. Background**

In numerous epidemiologic studies in the US and elsewhere, alcohol has not been associated with development or progression of chronic kidney disease. Whether this reflects potential cardiovascular benefits associated with moderate alcohol intake or competing risk of other causes of death, including liver disease and accidental deaths, is unknown. In theory, alcohol does exert a diuretic effect and could exacerbate volume depletion for individuals in an environment where they are vulnerable to volume depletion. Excessive alcohol consumption is associated with hyperuricemia, and there is suggestive evidence that hyperuricemia may adversely affect kidney function, both through local vasoconstriction as well systemic hypertension (Heinig and Johnson, 2006).

### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

Although we do not have data, we assume that alcohol is more likely to be consumed by men. We have no information regarding whether alcohol consumption is greater in Chinandega and Leon compared to other areas of the country.

Alcohol consumption has been assessed in 14 studies, and has been associated with a higher risk in eight of them (see Table 10i). Because of concerns about the specific effect of guaro lija (see below), studies that have looked at individual types of alcohol may provide better information. Overall, the epidemiological evidence for an association between alcohol and CKD in Nicaragua is not particularly strong.

### **c. Likelihood of causing CKD**

Based on multiple studies in different populations, alcohol consumption is not believed to cause CKD (Elsayed, 2007). However, it does contribute to volume depletion and so potentially could increase the susceptibility of the kidney to other exposures that cause CKD.

### **d. Likelihood of exposure**

Alcohol is known to be widely consumed.

### **e. Potential for study**

The only source of information on past and present alcohol use is self-report. Report of alcohol use may be susceptible to recall bias.

#### **f. Conclusions/Recommendations**

- i. The main evidence in favor of the alcohol hypothesis is the presumed higher consumption rate among men and its association with CKD in a number of studies.
- ii. We are not treating this as a high-priority hypothesis.
- iii. We will include questions on alcohol on questionnaires as the opportunity presents itself. Particular focus will be on alcohol consumption prior to intense work exposure.

### **11. Guaro lija**

#### **a. Background**

Based on our current understanding, which primarily comes from the U.S. CDC but has been corroborated in part by others, guaro lija (or simply “lija”) is a form of rum that is produced at a commercial distillery, presumably under appropriate and safe conditions, and then is shipped in bulk to small independent distributors and retailers where it is further processed and then sold in plastic bags to individual consumers [personal communication, telephone, CDC, 2005]. At the time of production, the rum is the same as that which is eventually sold in bottles but has a much higher concentration of ethanol (95%). “Lija” should not be confused with homemade alcohol (although there remains some confusion on our part on this matter). A CDC study asked about homemade alcohol and found consumption to be both uncommon (<5%) and unassociated with CKD.

It has been suggested that lija has an independent association with incident CKD other than simply being a form of alcohol, possibly due to the introduction of an unknown toxin somewhere in the chain between production at the factory and consumption by the individual. After production, the concentrated alcohol is shipped in bulk in storage containers, which appear to be new at the distillery but later may be transferred to used containers. There is concern that these containers may have been previously used to store pesticides and other chemicals and may retain a residue, and/or that some of the plastic material on the inside of the container is dissolving into the liquid, particularly as ethanol is an excellent solvent. The independent distributors and retailers dilute the concentrated alcohol with water and then package it in individual units (typically, plastic bags) for sale. Toxic adulterants might be used during this process. In 2006, there was an outbreak of poisoning in the departments of Leon, Chinandega, and Managua which was traced to the addition of methanol to lija (<http://www.paho.org/english/dd/Ped/nicaraguaMetanol.htm>). Although methanol itself does not cause CKD, it does demonstrate that an adulterant has been added on a widespread basis on at least one occasion.

#### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

Although we do not have data, we assume that lija is more likely to be consumed by men. We also have no information regarding whether lija consumption is greater in Chinandega and Leon compared to other areas of the country. An alternative possibility is that adulteration has been more likely to occur in this region, as was the case with the

methanol poisoning incident. Lija consumption would be expected to be more common among poorer segments of the population. Data from one study indicates that CKD is associated with low socioeconomic status, as measured by educational attainment (Brookline Sister City Health Committee, 2008).

All of the studies that reported on lija consumption (n=6) found moderate to strong positive associations (Odds Ratios=2.0-11.0) that, when reported, were statistically significant.

Lija consumption has been assessed in six studies, and has been associated with a higher risk in all of them (see Table 10i), with odds ratios ranging from 2.0 to 11.0. In some studies, lija but not other forms of alcohol was associated. The study in Chinandega by Alonso in 2002 found an overall elevated risk of CKD associated with alcohol consumption (unclear how defined) (OR=4.3), but only with lija (10.8) and not with bottled rum (0.8) or beer (0.3)

### **c. Likelihood of causing CKD**

Typical adulterants added to alcohol include methanol, diethylene glycol, and ethylene glycol. None of these adulterants are known to cause CKD, but both diethylene glycol and ethylene glycol, if ingested in sufficient quantity, can cause severe toxicity including acute kidney failure. However, there is both an animal study and a single case report of a 36-year-old chronic alcohol abuser that suggest the possibility that sublethal ethylene glycol poisoning might lead to CKD (Roberts, 1969; Nizze 1997). The exposure in the case report was the ingestion of ethylene glycol 3-4 times approximately 12 years prior to diagnosis. There have also been cases of lead nephropathy associated with ingestions of moonshine, thought to be due to the use of lead soldered containers, such as radiators, to distill the alcohol. If the lija is diluted with such adulterants, it could lead to chronic lead nephropathy.

Without knowing the identity of the pesticides that might form a residue or the nature of the plastic lining, we cannot assess whether they cause CKD.

### **d. Likelihood of exposure**

Lija is known to have been widely available and consumed. In addition, the problem of re-use of pesticide containers is well known in Central America. Furthermore, the episode with methanol poisoning demonstrates that the practice of adulteration has taken place at least once. It is possible that adulteration may have become more common in the 1990s. In the 1980s, the distribution network was centralized. In the early 1990s, distribution was decentralized and handed over to small private distributors, presumably resulting in less control over practices.

In 2004, U.S. CDC tested 47 samples of lija as well as 5 additional samples of commercial rum for isoamyl, methanol, ethylene glycol, diethylene glycol, and several heavy metals (chromium, antimony, cadmium, mercury, bismuth) (email communication (2/17/09)). Most of the samples exceeded the U.S. EPA Maximum Contaminant Level for drinking water, though the relevance of this measure for amounts of alcohol consumed per day is unclear. Many of the samples, including the commercial rum, had

particularly high levels of antimony, which is common in soil and thus might find its way into wooden storage barrels.

In response to the methanol poisoning incident, the sale of lija has been officially banned, though it is unclear to what extent its sale and consumption continues.

#### **e. Potential for study**

We lack detailed information on the manufacture, distribution, and sale of lija, including how frequently adulterants may have been used. In addition, most of the information we do have is secondhand. There are no samples of lija from earlier years, and it is unclear whether testing current samples of lija will provide additional information, because adulteration likely would have been episodic and perhaps more likely to have occurred in the past. There are no other sources of information on current or former exposure other than self-report, which limits our ability to assess past exposure. At this point, people may be reluctant to report lija use either because they are aware that it's a competing hypothesis to work-related exposures, or because it is illegal.

#### **f. Conclusions/Recommendations**

i. The main evidence in favor of the lija hypothesis is the presumed increased consumption among men and its repeated strong association with CKD in a number of studies. However, because of the difficulties in identifying a contaminant which is likely to have been present only historically and sporadically, it may be difficult to make much progress in investigating the potential role of lija consumption.

ii. It will be helpful to talk with Health Ministry officials, law enforcement personnel, and other contacts to better understand past and present practices regarding lija manufacture, distribution, sale, and consumption, as well as the potential value of testing of current samples.

## **12. Kidney stones**

### **a. Background**

Nephrolithiasis is a recognized cause of ESRD (Gambaro, 2001), but in a series of 1,391 patients initiating dialysis in France it accounted for only 3% of cases and half of those were related to chronic infection (struvite nephrolithiasis) (Jungers, 2004). Gillen et al used the NHANES database to examine the relationship of a history of kidney stones to eGFR and found a significant relationship, but only in those with a BMI >27 kg/m<sup>2</sup> (Gillen, 2005). In such subjects the risk of having an eGFR between 30-59 ml/min/1.73 m<sup>2</sup> in those with a history of stones was 1.87 that of those without such a history.

It is known that that stones occur more commonly in people who work at high environmental temperatures (Borghj, 1993) and that in the US there is an association between ambient temperature and risk for nephrolithiasis (Fakheri, 2009). Therefore

there may well be an increased risk for kidney stones and possibly related CKD in Nicaragua.

**b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

No studies have looked at the relationship of CKD and kidney stones in Nicaragua. However, it has been reported to us that half the patients with a diagnosis of CKD have evidence for renal calculi by ultrasound (personal communication Director, Hospital Gaspar Garcia Laviana) and in other conversations several observers remarked on the high prevalence of kidney stones in the Nicaraguan population, even those without CKD. The higher risk in workers at high environmental temperatures would be consistent with the pattern seen in Nicaragua.

**c. Likelihood of causing CKD**

Although it may cause CKD, it is an uncommon cause in temperate developed countries. We are unaware of evidence regarding its frequency in semi-tropical undeveloped countries. The presence of kidney stones may lead to greater exposure with nephrotoxic agents during the course of treatment; these include NSAIDs and potentially nephrotoxic antibiotics.

**d. Likelihood of exposure**

Hot temperatures and dehydration are known risk factors.

**e. Potential for study**

If ultrasound images or reports are available on patients with CKD, a systematic review would be quite feasible.

**f. Conclusions/Recommendations**

i. Although risk factors for stone disease are prevalent in the population of interest, kidney stones are considered a rare cause of symptomatic CKD. However, given the ease of identification from existing ultrasounds and the reported high prevalence in the CKD population there, it would be important to investigate.

ii. Existing reports and ultrasounds should be examined both from a population with CKD as well as an appropriate control group.

## **13. Structural kidney disease**

**a. Background**

Structural kidney disease encompasses a broad group of kidney diseases, both congenital and acquired, that are usually easily recognized with renal imaging using either ultrasound or computed tomography. In adults the most common congenital kidney disease, occurring in 1 in every 400-1000 live births, is adult polycystic kidney disease (PKD) with autosomal dominant inheritance (Grantham, 2008). Reduced eGFR often develops by age 40, with a median age of ESRD of 54 years old. For reasons that

are unknown, men have more rapid progression to ESRD than women. PKD is readily diagnosed by renal ultrasound with 96% of affected patients having at least three unilateral or bilateral cysts by age 30.

**b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

There is little evidence of structural kidney disease as an important contributor to CKD in Nicaragua. Although some epidemiologic studies suggest that family history may be a risk factor for CKD and many of the structural kidney diseases have an increased familial incidence because of a genetic component, there is little or no radiologic data to support such a high incidence of structural kidney disease. Given the availability of ultrasound imaging in Nicaragua, it would have been expected that structural abnormalities would have already been identified as an important cause, if they did exist. Furthermore, these diseases tend to be geographically dispersed and although they cluster in families, one would not expect them to lead to higher prevalence of CKD in a specific region, because of their relative rarity in the population.

**c. Likelihood of causing CKD**

These are well-known causes of CKD, but are unlikely to occur with sufficient frequency to account for the high prevalence of CKD in the country.

**d. Likelihood of exposure**

There is no reason to suspect a higher incidence of these diseases in the Nicaraguan population and, with the exception of PKD, they are an uncommon cause of CKD worldwide.

**e. Potential for study**

The majority of these diseases are readily identified on ultrasound imaging and a review of existing ultrasounds in patients with known CKD could easily establish the prevalence of these causes.

**f. Conclusions/Recommendations**

- i. Structural kidney disease is unlikely to account for the increased prevalence of CKD in Nicaragua, but their prevalence can be easily ascertained from a review of existing renal ultrasounds.
- ii. We plan on reviewing a sample of existing renal ultrasounds to estimate the prevalence of structural renal abnormalities in the population.

## **14. Diabetes**

**a. Background**

Diabetes is a major cause of CKD world wide, particularly in the developed world because of the worsening obesity epidemic. There are two types of diabetes. Type I diabetes is one of the most common chronic diseases of childhood. It is thought to occur because of autoimmune destruction of insulin-producing pancreatic cells, leading

to insulin deficiency. It has a bimodal age presentation, with peaks at four to six years and 10-14 years of age. Type II diabetes is commonly a disease of obese adults, typically beginning after the age of 40. It is being seen with increasing frequency in obese children, and now accounts for one-third of the cases of diabetes mellitus in childhood in the United States. The mean duration of diabetes before the onset of nephropathy is 12 years and diabetic kidney disease is heralded by the onset of microalbuminuria, followed by overt proteinuria, decreases in kidney function and progression to ESRD over a period of 5-10 years. Diabetic kidney disease eventually develops in 25-50% of patients with diabetes, although these rates may be substantially lower with strict blood pressure and glycemic control. No clear gender specific risk has been identified.

#### **b. Consistency with epidemiologic data and clinical patterns of CKD in Nicaragua**

Ten studies provide information on the prevalence of diabetes in this area of Nicaragua; these included six cross-sectional studies and four case-control studies (Table 13). All studies relied on self-report except the Brookline Sister City study in which nonfasting random glucose tests were conducted and a case of diabetes defined as a blood sugar level of  $\geq 200$  mg/dl. The prevalence of diabetes was generally low, ranging from 0-7.1% in five of the six cross-sectional studies. When these data were broken down by disease status, nearly all prevalence rates were low among cases and non-cases (e.g., 0-9.4%).

Exceptions were observed in three studies reporting prevalences ranging from 15.4-27.3%. Higher rates were observed in an urban population of Chichigalpa (27.3%), among stage 4 cases in La Isla and Candelaria (23.4%), and among cases (23.1%) and controls (15.4%) recruited from a hospital in Leon. Sample sizes were small in most of these settings, perhaps accounting for the atypical results.

The generally low prevalence of diabetes, even among cases of CKD, suggest that, while diabetes is an important risk factor for developing CKD on an individual level (Table 10f), it likely accounts for only a small portion of the excess occurrence of CKD at the population level. The reported absence of significant proteinuria in patients with CKD would also argue against diabetes as an important causal factor.

#### **c. Likelihood of causing CKD**

Diabetes is a well-known cause of CKD and the most common cause in many developed and developing countries.

<b>Table 13. Estimates of Diabetes Prevalence</b>				
<b>Author and Year</b>	<b>Location</b>	<b>Overall Prevalence (%)</b>	<b>Prevalence in CKD Cases (%)</b>	<b>Prevalence in Non-Cases (%)</b>
<b>Cross-sectional Studies</b>				
Callejas Callejas, Alonzo Medrano, Mendoza Canales 2003	Leon and Chinandega	3.0	5.0	2.8
Callejas Callejas, Alonzo Medrano, Mendoza Canales 2003	Chinandega	1.8	8.3	1.3
Alonso, Callejas Callejas, Dominiguez Moya 2003	Chinandega	7.1	9.4	4.8
Torres, Gonzalez, Vanegas, Aragon 2008	Chichigalpa	0 (rural area) 27.3 (urban area)	NA	NA
Torres Lacourte, Gonzalez, Vanegas, Aragon 2008	La Isla and Candelaria	0.5 (La Isla) 1.3 (Candelaria)	23.4 in stage 4 cases	NA
Aragon, Torres Lacourte, Gonzalez 2008	Northeast Leon	1.1	0	NA
<b>Case-Control Studies</b>				
Castrillo, Bonilla, Estrada 2001	Leon	NA	23.1	15.4
Alonzo Medrano, Perea 2002	Chichigalpa	NA	0	2
Callejas Callejas, Alonso Medrano, Mendoza 2003	Chinandega	NA	0	0
Sister City Health Committee 2008	Quezalguaque	NA	6.0 (random glucose test)	4.4 (random glucose test)

NA not available

#### **d. Likelihood of exposure**

As discussed, diabetes is uncommon in the population, so diabetic nephropathy would also be uncommon.

#### **e. Potential for study**

Diabetes is readily diagnosed from simple blood and urine tests and in most patient diabetic nephropathy is initially diagnosed by the presence of proteinuria on urine dipstick. Existing records of patients with CKD should provide a reasonable estimate of the prevalence of diabetes in the CKD population, although it may provide a slight underestimate since mild cases may have gone undiagnosed and diabetic symptoms may diminish as renal function worsens.

## **f. Conclusions/Recommendations**

1. Based on available data, diabetes does not appear to be a major cause of CKD in sugar cane workers. However, because diabetes is a well-known and common cause of CKD, its prevalence in the affected population should be estimated.
2. Review of medical records would provide the most efficient means of providing this estimate and should have reasonable reliability.

## **15. Hypertension**

### **a. Background**

Hypertension is both a common cause of kidney disease and a consequence of CKD. Long-standing uncontrolled hypertension is a well-known cause of CKD and, over a period of 14-18 years, 1-3% of such patients will develop end stage renal disease. Because hypertension is highly prevalent in developed societies, it is an important cause of CKD accounting for 25-40% of cases. However, hypertension also occurs as a complication of CKD. Approximately 80-85% of patients with CKD will have hypertension and the prevalence of hypertension increases as the eGFR falls, so that at an eGFR of 15 ml/min/1.73 m<sup>2</sup>, 95% of patients will be hypertensive. A number of studies have shown that control of hypertension is an important factor in reducing the rate of progression of CKD.

### **b. Consistency with epidemiologic data and clinical patterns of CKD in Nicaragua**

Available data do not suggest that hypertension is a major causal factor in the CKD epidemic in Nicaragua. Because the onset of essential hypertension is typically at age 30-50 years and progression to CKD occurs over 10-20 years, the age of onset of CKD is typically 40-60 years, older than what has been described in Nicaragua. According to reports from physicians in Nicaragua, hypertension is often not seen in early CKD, but as expected occurs later in the disease. Hypertensive renal disease is felt to occur equally in both sexes and would not explain the male predilection for CKD in Nicaragua.

### **c. Likelihood of causing CKD**

A well-accepted cause of CKD.

### **d. Likelihood of exposure**

One difficulty for determining causality for patients who have not received prior medical care and who present with renal dysfunction and hypertension, is that it is usually impossible to determine whether the CKD has occurred as a consequence of uncontrolled hypertension, or whether the hypertension is a complication of the CKD. One would have to determine the prevalence of hypertension in a non-affected population (without CKD) to determine whether it could be considered a causal factor.

### **e. Potential for study**

Record review would only be useful if blood pressure recordings are available prior to the onset of CKD. If prevalence data in a population without CKD are available, they would aid in assessing the attributable risk due to hypertension, since there would need

to be a high prevalence of hypertension prior to the onset of CKD to account for high risk of CKD.

#### **f. Conclusions/Recommendations**

1. Although hypertension is a common cause of CKD in developed countries and is an exacerbating factor in CKD progression, it does not appear to be a common primary cause of CKD in Nicaragua.
2. A medical record review should be done to determine the prevalence of hypertension in the population. In particular, patients with incident CKD should be identified, so that the prevalence of hypertension prior to the development of CKD can be identified.

## **16. Glomerulonephritis**

### **a. Background**

Glomerulonephritis is a common cause of CKD world wide and in some countries, such as Japan, is the major cause. It encompasses a number of different diseases, including some with systemic manifestations, and some confined to the kidney. Glomerulonephritis has a broad age distribution with different peak incidences for the different specific diseases. Depending on the specific cause, it may present with acute kidney injury (acute glomerulonephritis) or with chronic kidney disease. Urinalysis is a critical component for arriving at a specific diagnosis. Based on the urinalysis, nephrologists broadly divide this group of diseases into those presenting with nephrotic syndrome and those presenting with nephritic syndrome, although there is some overlap. Nephrotic syndrome is characterized by high-grade proteinuria with 4+ proteinuria (albuminuria) on urine dipstick and greater than 3.5 g/d of proteinuria on quantitative determinations. The nephritic syndrome is characterized by the appearance of red blood cell casts in the urine sediment. Those glomerulonephritides presenting as nephrotic syndrome will often have a progression to ESRD over years. In contrast, the diseases presenting as nephritic syndrome will often have an acute course and may present with acute renal failure with progression to ESRD over days or weeks if not appropriately treated. Some of the glomerulonephritides appear to have an increased incidence in certain geographic areas, including an increased incidence of IgA nephropathy in Japan and membranous nephropathy in China (thought to be a manifestation of the high prevalence of hepatitis B in China).

### **b. Consistency with epidemiologic data and clinical patterns of CKD in Nicaragua**

There is no wide-spread evidence for high grade proteinuria in the early stages of CKD in the Nicaraguan population. Although cases of secondary nephrotic syndrome may be due to infections such as hepatitis B and C, the prevalence of these infections is not known to be markedly increased in the population.

### **c. Likelihood of causing CKD**

They are known causes of CKD, but are more common as a sporadic cause of CKD.

#### **d. Likelihood of exposure**

There is no reason to suspect an increased risk in the population due to a high prevalence of hepatitis B or C.

#### **e. Potential for study**

If glomerulonephritis were an important cause of CKD in the population, it would be expected that the majority of patients would have 4+ proteinuria on urine dipstick early in the course of their CKD and the proteinuria also tends to persist even at late presentation. A review of medical records for the presence of high grade proteinuria would provide a ready means of assessing the relative contribution of this etiology.

#### **f. Conclusions/Recommendations**

1. It is unlikely that glomerulonephritis is an important contributor to the increased prevalence of CKD in the study population.
2. Review of medical records for the presence of high grade proteinuria, provides a simple means of estimating the prevalence of glomerulonephritis.

## **17. Urinary Tract Infection**

#### **a. Background**

In multiple studies originating from Nicaragua, including data gathered in the Quezalguaque Sister City project (not included in the table), urinary tract infection (UTI) has been associated with prevalent CKD. In the US, UTI is an uncommon cause of CKD and kidney failure, with individuals with kidney disease due to UTI typically developing recurrent infections in early childhood due to structural abnormalities, including posterior urethral valves and ureteropelvic junction obstruction. Vesicoureteral reflux is defined as the retrograde passage of urine from the bladder into the upper urinary tract. It is the most common urologic anomaly in children and may predispose to recurrent UTIs. Severe pyelonephritis and recurrent UTIs have been associated with subsequent renal scarring, but this is an unusual cause of kidney failure.

#### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

Ten studies examined a history of urinary tract or kidney infections (Table 11e). In some studies exposure was defined as recurrent infections. The results of these studies were inconsistent; six found modest to large positive associations (Odds Ratios=1.4-5.4) while four had essentially null findings. Three of the six positive studies had statistically significant findings. However, only a few of the studies controlled for confounding variables. The two studies that controlled for at least sex and age found 1.4-fold and 3.2-fold increased risks (Castrillo, 2001; Lopez Arteaga, 2005).

The major difficulties with this data are the definitions and ascertainment of UTI, both by the individual who may be relating a history of UTI as well as by the physician who may have provided the individual with the diagnosis. Based on personal communication with

community residents and physicians during the fact finding trip, in some cases, UTIs are diagnosed simply based on flank pain or dysuria, and there is little in the way of conclusive evidence. Often this would lead to the use of prescription medications, including antibiotics (per the workers) or the use of herbal remedies (per the physicians' report). Accordingly, the question is raised, assuming that there is an association between an individual recalling a history of UTI and prevalent kidney disease and that this is not driven by an attribution bias, whether a treatment for "UTI" could be implicated. Finally, sexually transmitted diseases including gonorrhea and Chlamydia are a far more common cause of dysuria in adult men than UTIs. These STDs are not associated with CKD.

**c. Likelihood of causing CKD**

UTIs are an unusual cause of kidney failure.

**d. Likelihood of exposure**

UTIs are extremely common in all pediatric populations, particularly girls, and remain common in adult women. UTIs are exceptionally unusual in adolescent boys and adult men.

**e. Potential for study**

Could be addressed in a study of children when there is less recall bias; however, the utility of this is low.

**f. Conclusions/Recommendations**

- i. Low priority for study, with the exception of questions regarding medication administration and evaluation of medication administration for flank pain and dysuria in adult male workers.
- ii. Conduct qualitative interviews with physicians on diagnostic practices regarding UTI and consequent prescription of nephrotoxic medications.

## **18. Genetics and CKD**

**a. Background**

Multiple lines of evidence suggest that the susceptibility to develop CKD has a significant genetic component. There are a number of specific forms of renal disease that are caused by mutations to a single gene including polycystic kidney disease, Alport syndrome, some forms of focal segmental glomerular sclerosis, and some forms of tubulointerstitial disease (including those associated with a mutation in the uromodulin gene (UMOD) which is linked to medullary cystic kidney disease). In addition, there is now extensive evidence that suggests that renal disease in the general population has a genetic component. Multiple approaches have been employed in an effort to identify these genes with limited results.

In the candidate gene approach, specific genes are selected for analysis based on a

function or a gene product that is closely related to renal disease. Since 1999, over 100 articles have been published on candidate genes in CKD. This method is very sensitive but determining the relative importance of the candidate gene and independently replicating the results of the studies is difficult. These studies are also challenging because of the late age of onset and because linkage analysis has suggested that there are no genes of high penetrance (> fourfold increased risk) in populations of European descent (Fox, 2004; Hunt, 2004). If the pattern of kidney injury in the Nicaragua population was consistent with a kidney disease with an established genetic pathophysiology, the candidate gene approach might be able to confirm a common mutation.

The genome screen approach is more difficult, time consuming, and expensive but has the advantage of being able to locate new and as of yet undiscovered genes. Approximately 400 genetic markers that identify evenly spaced locations along each of the human chromosomes are genotyped in families with multiple cases of renal disease. Linkage analysis determines which parts of which chromosomes are being inherited in renal disease. This technique led to the discovery of MYH9 (Kao, 2008), a gene linked to an increased risk of CKD in African Americans. This technique relies on the development of an admixture map to identify evenly spaced locations along the chromosome and is very time consuming and expensive.

#### **b. Consistency with demographic patterns and epidemiologic data regarding CKD in Nicaragua**

Genetic diseases would be expected to have a familial clustering, and although there has been some suggestion of an increased risk for CKD in families, it is uncertain how widespread the family clustering is in Nicaragua. The male predominance might also be consistent with an X-linked recessive trait, but such diseases are transmitted to male progeny by the mother and therefore typically skip generations. However as previously noted the autosomal dominant polycystic kidney disease seems to progress more rapidly in males and although, as already discussed an increased prevalence of polycystic kidney disease is unlikely, it is conceivable that some other genetic disease with differential expression in males and females exists. Of note, there are autosomal dominant tubulointerstitial kidney diseases that may present with ESRD in the fourth and fifth decades of life, following the type and course of CKD seen in Nicaragua, but in the absence of strong family clustering, these genetic diseases would be unlikely (Bleyer, 2009)

#### **c. Likelihood of causing CKD**

In the absence of strong family clustering it is unlikely that CKD in Nicaragua is due to a single gene mutation. However, it is possible that a genetic factor, rather than being etiologic, increases the susceptibility of the population, such as has been described for the MYH9 allele and the risk of ESRD in African-Americans.

#### **d. Likelihood of exposure**

Unknown.

**e. Potential for study**

Genetic analysis would be prohibitively expensive and unlikely to be of high yield with current techniques. A prospective study investigating familial clustering might identify the possibility of single gene mutations, but existing evidence does not suggest strong familial clustering, so such a study would be low yield. The cost of genetic analyses is decreasing markedly with time and it is relatively inexpensive to collect and store DNA for future study. However, such studies would likely occur beyond the time frame of the planned project.

**f. Conclusions/Recommendations**

i. A monogenic etiology for the prevalent CKD in the population is highly unlikely. Although there may well be genetic susceptibility factors, identifying such factors is costly, difficult, and unlikely to have an immediate impact on the at risk population.

ii. No genetic analytic component is planned in the short term but we recommend storing sera for subsequent genetic testing should familial clustering be noted or a hypothesis suggesting genetic predisposition become more likely.

## **IV. RECOMMENDED ACTIVITIES**

### **A. Introduction**

The preceding sections demonstrate that CKD is clearly a problem in northwestern Nicaragua with characteristics that are unusual in relation to typical patterns of CKD. Furthermore, the studies conducted to date have been more successful at defining the nature of the problem and identifying hypotheses than at identifying its causes. The reasons for the failure to make more progress include lack of resources and coordination, lack of access to important sources of data, as well as what appears to be the inherent complexity of the problem, including the possibility of multiple interacting factors.

An additional level of complexity is introduced by the need to address historical as well as current exposures. In part, this is because the possible induction and latency periods between exposure and disease diagnosis are unknown and could encompass a wide range. Equally important, it is not hard to imagine that exposures may have changed whether related to occupational or non-occupational factors. On the one hand, an understanding of current factors that are causing CKD is important for purposes of designing interventions to prevent future deaths. At the same time, the complaint submitted to the World Bank was on behalf of workers whose median year of diagnosis was 2002 based on data collected by ASOCHIVIDA from its members. Even if analysis of current exposure finds no association with work practices, it does not mean that they could not have played a role at a previous time.

In the previous section, we tried to take a step back and review all the hypotheses that might need to be considered based on knowledge about general causes of CKD as well as less well-established factors that might be operating in Nicaragua. The dilemma that becomes clear from the review of these hypotheses is that the exposures that large numbers of people (but predominantly men) in northwestern Nicaragua are likely to experience are not clearly associated with CKD, while those factors that clearly cause CKD are not likely to be sufficiently common and/or should have an exposure pattern that does not correlate with the relative frequency of disease.

As a result, we have concluded that it is appropriate to propose a set of the following nine activities that at least touch on the entire range of hypotheses, while at the same time focus primary attention and resources on those areas that we have deemed to be highest priority based on both concerns of the parties and our own assessment:

1. Environmental sampling
2. Analysis of biological samples
3. Work observation
4. Cohort study
5. Review medical records

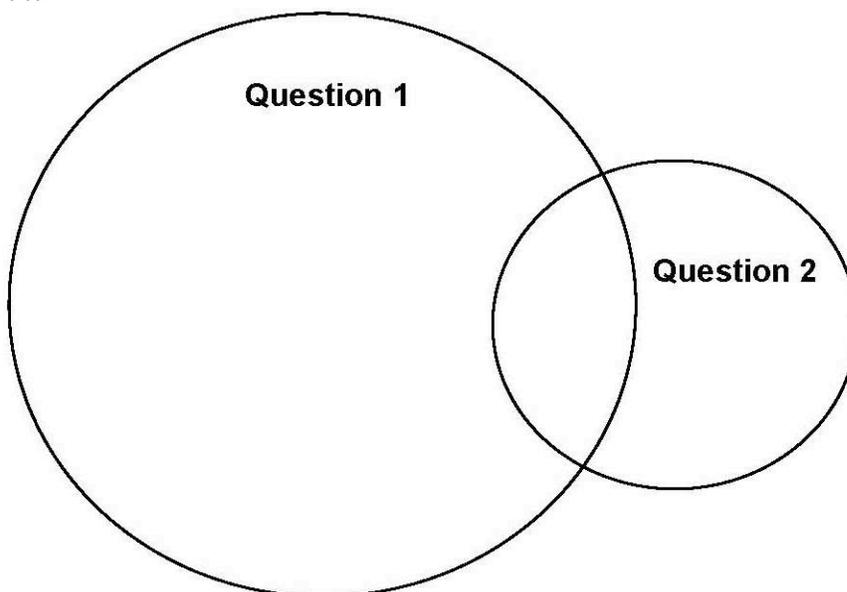
6. Urinalysis in adolescents
7. Postmortem biopsies
8. Key informant interviews
9. Other possible activities

In order to more fully elaborate the rationale for selecting these activities, it is important to return to the original mandate from the Dialogue process, which was to develop recommendations for activities that could lead to an answer to the following two questions:

1. What are the causes of CRI in the Western Zone (Zona del Occidente) of Nicaragua – an area that includes the Ingenio San Antonio and its sugarcane plantations?
2. Is there any relationship between the practices of the Ingenio San Antonio and the causes of CRI?

These questions overlap but are not identical; for example, it is possible to come to a determination regarding occupational exposures without determining the causes of CKD in the region (Figure 7). In addition, activities directed toward answering the question regarding NSEL's responsibility can be more focused and modest in scope -- and therefore less expensive and time-consuming -- than a set of activities framed by the first question. Finally, although we stress that science and the search for causality always involves uncertainty, there is a much greater chance that a reasonable set of activities can result in being able to draw conclusions regarding the likelihood that practices at ISA have or have not contributed to the occurrence of CKD among its workers than would be the case for the broader causal question.

**Figure 7. Relationship of questions identified by participants in Dialogue process, February 2009**



Meanwhile, as the Dialogue process has developed, it has further clarified the following:

- (1) the question of the company role is critical to making progress among the parties;
- (2) the timeframe must be as short as possible consistent with the requirements of good scientific methodology; and
- (3) funds are not unlimited.

For all these reasons, our recommended activities are primarily aimed at answering the question related to occupational practices, with most of our resources going toward addressing the associated key hypotheses: exposure to agrichemicals and the sequelae of volume depletion and/or muscle damage brought on by strenuous work in stressful environmental conditions.

A number of the activities recommended in this section are not directly related to occupational exposure, for example, urinalysis among adolescents. However, we concluded that they were important to include for at least three reasons. First, and most importantly, we must address the possibility that both occupational and non-occupational factors are interacting in a synergistic manner to greatly increase the risk of CKD. These include non-occupational factors that might themselves not fit the exposure pattern expected based on the epidemiologic characteristics of CKD in Nicaragua but which when combined with an occupational exposure could produce the observed distribution of disease. Second, we have the opportunity to test for the presence of established causes of CKD that have never been assessed and for which another opportunity may not arise for a long period of time. Therefore, some of the activities proposed, such as environmental and biological testing of aristolochic acid and the components of volcanic emissions (heavy metals), are intended to screen for the presence of these substances. If they are not present in any meaningful quantity, these hypotheses can be set aside. However, if they are present, it would be more than unfortunate to have missed the opportunity to identify the cause of the epidemic due to the failure to pursue these possibilities. Finally, if the result of our work is that we find no association between occupational practices and CKD among workers at ISA, both we and others will be more confident that the results are accurate rather than due to problems with the study if a plausible alternative is identified, even if not definitively.

In carrying out these recommendations, we will also need to maintain flexibility. Early findings may lead us in directions that we have not elaborated here. For example, if we were to find evidence of substantial exposure to heavy metals or aristolochic acid or evidence of early signs of renal damage among adolescents prior to starting to work, then it would be incumbent upon us to adjust our plans to allow us to follow-up those findings with studies to assess their potential effect on CKD in the region.

One activity we have not proposed is another prevalence study in the region. Although it would provide useful information, a number of concerns led us to conclude that it would

not be the most productive use of resources. First, neither the costs nor time involved in mounting such a study are trivial. Particularly because it would require postponing many of the other activities, it would also mean possibly adding a year to the study duration. Second, as described previously, a number of studies have already been conducted, and while they are not perfect, there does not seem to be a strong probability that a new study -- even if more rigorous -- will come to drastically different conclusions. In addition, other groups are currently conducting or just beginning studies that can provide comparable information (see Section IV.B.9, Other Possible Activities). Finally, as with the prior studies, it is difficult to imagine how an additional prevalence study would obviate the need to conduct analytic studies that can truly assess exposure and test for associations. This is particularly true given that a prevalence study can obviously only assess current patterns of disease, but we must also assess associations based on past exposure and disease.

Although not specific recommendations, key elements of a successful study will be frequent and open communication with the Dialogue parties and collaboration with Nicaraguan agencies (e.g., MINSA) and organizations that have been active in trying to find solutions and which have a responsibility for this problem. As an example of communication, we have worked with ASOCHIVIDA Board members to develop their understanding of the goals, approach, and methods of research, which are not obvious to persons who have not been trained in this area (see Appendix). The goal was to give them the tools to appreciate the reasoning behind our recommendations and to be able to ask critical questions. Going forward, even though we estimate the study will not be completed for two years, interim results from different activities will begin to be available much sooner. We will keep the Dialogue members apprised of our progress as well as our difficulties on an ongoing basis and seek their counsel.

Another key element of a successful study will be to solicit and receive input from other scientists. There is too much at stake in this study for our plans and activities not to be scrutinized by outside reviewers. The typical mechanism for providing this input in a research study is a Scientific Advisory Board (SAB), which is composed of a group of researchers chosen for their expertise in different areas relevant to the study. Preliminarily, we propose a SAB comprised of four members, two from Nicaragua or elsewhere in Central America and two from the U.S., Canada, or Europe, which would meet two times a year. The SAB would review study designs and protocols, and substantive changes in designs and protocols, as well as review issues related to implementation such as recruitment; data collection, processing, and analysis; sample collection, storage, processing, and analysis; and ethical concerns.

## **B. Specific Recommendations**

There are three categories of activities that we recommend with respect to investigating environmental exposures and working conditions in the northwestern region of Nicaragua. The first category is environmental sampling, which would include the collection of soil, drinking water, and food. The second category is biological sampling, which would include the analysis of metals, aristolochic acid, and potentially selected agrichemicals in blood, urine, or other appropriate sources. The third category is to perform work observations, which would include an assessment of industrial hygiene practices and working conditions, particularly with respect to volume depletion.

We recognize that obtaining agreement on the final details of all study design elements (i.e. sample locations, etc) prior to implementation is an essential component of our effort if we expect all stakeholders to accept the eventual findings. Accordingly, all design elements will be discussed with representatives of NSEL and ASOCHIVIDA prior to implementation and a representative from each group will always be welcome to accompany the field team. Each of the three categories of activities is described in the subsections below.

### **1. Environmental Sampling**

In the northwestern region of Nicaragua, the extent to which surface soil may be contaminated with metals or agrichemicals has not yet been explored while comparable investigations of drinking water have been limited. Similarly, the presence of metals or aristolochic acid in food has not yet been investigated. We therefore propose to collect samples of surface soil, drinking water, and food and analyze these samples for agrichemicals, metals, and aristolochic acid as appropriate.

However, while metals are known to be toxic to the kidney and easily detected in environmental samples, the details surrounding agrichemicals are more complex. Accordingly, we recommend that the first step should be to investigate the toxicology and physical/chemical properties of 20 agrichemicals for the purpose of finalizing an appropriate list of analytes, prior to conducting the very expensive analysis of agrichemicals in environmental samples (Section IV.B.1.a). The remaining subparts of Section IV.B.1. address the collection and analysis of surface soil (Section IV.B.1.b), drinking water (Section IV.B.1.c), and food samples (Section IV.B.1.d).

#### **a. Assessment of Agrichemicals**

Both NSEL and ASOCHIVIDA representatives have expressed the desire for a systematic evaluation of pesticide exposures and their possible association with CKD. There is concern not only about current and past exposures to agrichemicals used

during the production of sugarcane, but also about potential exposure to agrichemicals that were used historically in the production of other crops such as cotton.

NSEL has provided our team with a list of agrichemicals that have been used on their sugarcane fields. This list includes: Ametrex (Ametryne CAS#834-12-8), Finale (Glufosinate ammonium CAS#77182-82-2), Karmex (Diuron CAS#330-54-1), Hedonal (2,4-D CAS#94-75-7), Prowl (Pendimethalin CAS# 40487-42-1), Roundup (Glyphosate CAS#1071-83-6), Forza (Glyphosate CAS#1071-83-6), Misil II (Metsulfuron methyl CAS# 74223-64-6, Dimethylamine Dicamba CAS#2300-66-5), Advance (Trifluralin CAS#1582-09-8), Velpar 75 (Hexazinone CAS#51235-04-2), Racumin (Coumatetralyl CAS#5836-29-3), JADE (Imidacloprid CAS#138261-41-3), and Atrazine (CAS# 1912-24-9). Additionally, representatives from NSEL have mentioned Nemagon (dibromochloropropane CAS#96-12-8) and DDT (CAS# 50-29-3) as additional agrichemicals that were used historically in the region by industries producing other crops such as peanuts, bananas, and cotton.

Similarly, the ASOCHIVIDA board members have also provided a list of agrichemicals that they believe were used by NSEL. This list includes: Roundup (Glyphosate CAS#1071-83-6), Velpar 75 (Hexazinone CAS#51235-04-2), Ametrex (Ametryne, CAS#834-12-8), Karmex (Diuron CAS#330-54-1), Gramoxeone (Paraquat CAS#1910-42-5), Terbugran (Terbufos CAS# 13071-79-9), Coumadin (Warfarin CAS# 81-81-2), Arrivo (Cypermethrin CAS# 52315-07-8), Atrazine (CAS# 1912-24-9), and Prowl (Pendimethalin CAS# 40487-42-1).

As an external check on the list of agrichemicals that may have been used in sugarcane production, we consulted the PAN-Pesticides Database which catalogs pesticide use in California (PAN-Pesticides Database, 2009). According to that database, the agrichemicals atrazine, glyphosate, pendimethalin, and 2,4-D represent 99% of the total pesticides applied to sugarcane in California in 2007 (the last year for which data are currently available). These data consider the total pounds of the active ingredients applied to sugarcane but do not include inert ingredients. All four of these agrichemicals were included on the list provided by NSEL.

#### **i. Assess the Completeness of Agrichemical list**

The list described above includes 20 agrichemicals that may have been used in the region and to which NSEL workers may have been occupationally or environmentally exposed. To ensure that there are no key omissions, we plan to consult with various other sources such as the Nicaraguan Ministry of Agriculture, other sugarcane producers, and agrichemical suppliers to determine if there are other agrichemicals that may have been applied in this region. Additionally, to the extent that the information is available, we will collect data on the amounts and years of application for each agrichemical.

## **ii. Toxicological Review of Agrichemicals**

The analysis of agrichemicals is very expensive and to analyze for all of the chemicals on our list could cost \$800-1100 per sample. Accordingly, prior to analyzing surface soil and drinking water samples for these 20 agrichemicals, we recommend that a toxicologist review the literature and determine whether there is sufficient evidence to suggest that each could be associated with CKD.

Based on our initial review, it appears that only a few of the agrichemicals that may have been used by NSEL have been shown to be associated with effects on the kidney: glyphosate (USEPA, 2009a), paraquat (CDC, 2009; USEPA, 2009b), and atrazine (ATSDR, 2009; USEPA, 2009c). However, given the focus on agrichemicals as a primary hypothesis of concern by representatives of both NSEL and ASOCHIVIDA, we want to be cautious about excluding potential contaminants of concern and therefore recommend that a more thorough review of the literature be conducted by a toxicologist.

Based on the literature review, each agrichemical (considering both active and inert ingredients) will be categorized into one of three groups: (1) Agrichemicals with evidence of kidney effects, in cases where the chemical has been linked to kidney damage, (2) Agrichemicals of potential concern, in cases where kidney effects have not been studied or in which the mechanism of action would be consistent with kidney damage, and (3) Agrichemicals with no evidence of kidney effects, in cases where kidney damage was investigated as an outcome of interest but no effects were observed, or in cases where the mechanism of action is not consistent with kidney damage.

## **iii. Assess Relevance of Environmental Sampling**

As the next step in assessing all 20 agrichemicals, we will review the physical and chemical properties to better understand how each would be expected to behave in the environment, as well as the toxicokinetics to better understand how each would be expected to behave in humans. For instance, agrichemicals with short half-lives in the environment would only be expected to be found in surface soil or drinking water if they were applied very recently, whereas agrichemicals with long half-lives would be more likely to be detected in surface soil or drinking water even if applied years earlier. Understanding how each agrichemical behaves in the environment will be a key component of determining whether sampling surface soil and/or drinking water is a feasible option for assessing exposure to agrichemicals.

Similarly, we will review the toxicokinetics to better understand how each would be expected to behave in humans. For instance, some agrichemicals might be most easily measured in urine samples and represent exposure during the past 24 hours, whereas others might be more easily measured in blood samples and represent exposure over a longer period of time. Understanding how each agrichemical behaves in humans will be

a key component of determining whether biological sampling is a feasible option for assessing exposure to agrichemicals.

#### **iv. Finalize List of Analytes by Sample Type**

The ultimate objective of this step is to finalize a list of agrichemicals that may cause kidney damage and determine whether it is feasible to assess current and/or historical exposure via analysis in different types of samples. The results of this important step will be used to identify a final list of agrichemicals to analyze in surface soil and drinking water samples, as well as potentially in biological samples during a subsequent phase (not discussed in this report). Perhaps most importantly, we will present this final list of agrichemicals to representatives from NSEL and ASOCHIVIDA and provide the necessary background information as a rationale for inclusion or exclusion. We will seek to obtain mutual agreement on the agrichemicals to be tested before conducting any analyses.

#### **b. Surface Soil Samples**

The extent to which surface soil may be contaminated with metals or agrichemicals has not yet been explored in the northwestern region of Nicaragua. Agricultural workers are likely to have relatively high levels of exposure to contaminants in surface soil via both incidental ingestion and inhalation of fugitive dust. Similarly, given the presence of exposed surface soil both indoors and outdoors at many residential properties, residents in this region are also likely to have exposure to contaminants in surface soil via both incidental ingestion and inhalation of fugitive dust.

As a first step toward evaluating the potential role of contaminated surface soil, we propose to collect approximately 200 surface soil samples from sugarcane fields and communities throughout the region. This phase of sampling is not intended to provide a comprehensive characterization of surface soil contamination, but is rather proposed as a screening level effort to assess the potential human health risks associated with occupational and residential exposure to metals (and potentially agrichemicals pending the results of the effort described above in Section IV.B.1.a) in surface soil via incidental ingestion and inhalation of fugitive dust.

#### **i. Data Collection**

We propose collecting a total of approximately 200 surface soil samples from five categories of agricultural fields and from five different residential areas, all in the northwest region of Nicaragua. The five categories of agricultural fields include:

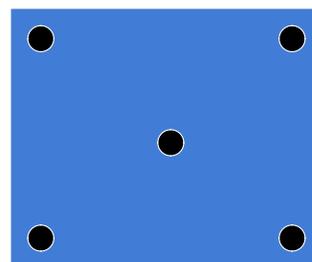
- ◆ fields at the ISA that have never been used for crops other than sugarcane (20 samples)
- ◆ fields at the ISA that are currently used for sugarcane but previously used for other crops (20 samples)

- ◆ fields owned by private landowners but leased and operated by NSEL for the production of sugarcane (20 samples)
- ◆ fields neither owned nor operated by NSEL for the production of sugarcane (20 samples)
- ◆ fields neither owned nor operated by NSEL for the production of crops other than sugarcane (20 samples)

Samples will also be collected from five residential communities that differ according to primary industry of employment, which would be expected to be associated with different prevalences of CKD. We plan to include La Isla and Candelaria because current and former workers at ISA and their families comprise the majority of residents. The remaining three communities have yet to be selected but would differ in primary industry and include few or no ISA workers. We propose collecting 20 outdoor surface soil samples from each of the five communities.

The exact sampling locations within each of category of agricultural fields and within each community will be selected to provide a modest spatial assessment of each area and a portable global positioning system (GPS) will be used to record the exact coordinates of each sample location. The samples collected from the communities will be collected on residential properties but outside the homes or other structures (ie from the yards).

At each of the 200 sample locations, sticks, rocks, or other debris will be removed from each area and soil samples will be collected at depths from 3-6 inches using disposable plastic scoops. For each sample, approximately equal sized amounts of soil will be collected from five composite points within a 10 by 10 square foot area (in a pattern as shown at right) and mixed in a plastic Ziploc bag. Two aliquots of the mixture will be placed in clean labeled sample containers, one to be analyzed for metals via inductively coupled plasma mass spectrometry (ICPMS), and the other to be stored at -20°C for possible future analysis of agrichemicals. Soil samples will be sieved to 250 microns prior to analysis to ensure that the measured fraction is most relevant to exposure. One field blank and one duplicate will be collected for each category of agricultural fields and in each community (total of 10 field blanks and 10 duplicates).



## ii. Data Analysis

The descriptive analysis of the surface soil data will be conducted using summary statistics, graphical displays, and correlation coefficients. Shapiro-Wilks tests and graphical displays will be used to determine the nature of the distribution of the metals and agrichemicals in surface soil. For the purposes of statistical analyses, a log transformation of the data will be applied if necessary to produce an approximately normal distribution.

Method limits of detection (LOD) will be estimated as three times the standard deviation of the field blanks. In cases where the mean field blank amounts are significantly

different from zero ( $\alpha=0.05$ ), the corresponding data will be corrected by subtracting the mean field blank amounts from the sample amounts. Units for metals and agrichemicals in soil will be reported as mg/kg and all statistical analyses will be conducted using SAS statistical software with statistical significance reported at the 0.05 level.

Since metals occur naturally in the environment, we will investigate whether background data are available for surface soil in Nicaragua but will at least compare to previously reported background levels in other countries (Kabata-Pendias, 2001). Linear regression models will be used to determine whether contaminant levels are significantly different by microenvironment. For instance, are levels higher in the agricultural fields compared to the residential areas? Are levels different among the different categories of agricultural fields or among the different residential communities? To the extent that CKD prevalence data are available for each of the residential communities, we will investigate whether contaminant levels by community are generally consistent with the observed differences in CKD prevalence.

An analysis of potential health risks associated with metals and agrichemicals in surface soil will be conducted in accordance with the USEPA's risk assessment guidance for human health evaluation activities (USEPA, 1989). Exposure concentrations in surface soil will be combined with exposure factors that are representative of the intensity and frequency of exposure among agricultural workers in Nicaragua. These assumptions regarding contact rate, frequency, duration, and body weight will be used to estimate contaminant intake (mg contaminant/kg bodyweight/day). Toxicity information (ie. RfDs, critical effects, uncertainty and modifying factors) will be obtained from USEPA's Integrated Risk Information System (IRIS) and combined with the intake estimates for the purpose of characterizing the potential risks associated with occupational and residential exposure to contaminants in surface soil.

### **c. Drinking Water Samples**

The extent to which drinking water may be contaminated with metals or agrichemicals has received little attention in the northwestern region of Nicaragua. While NSEL has indicated that groundwater samples have been monitored from onsite wells, these samples collected at depth may not be representative of water that is actually consumed by workers (Pollutech, 2001). A summary of the previous investigations that have analyzed contaminants in water samples is provided in the Appendix.

It has been reported that sugarcane workers, at least historically, have obtained their drinking water from both wells and from surface water. It is also our understanding that in the communities, residents obtain their water from individual wells as well as from common sources that are piped to individual properties. We propose to collect water samples from the exact locations where the workers and residents obtain their drinking water.

As a first step toward evaluating the potential role of contaminated drinking water, we propose to collect a maximum of 200 drinking water samples from sugarcane fields and

communities throughout the region. Similar to the surface soil sampling, this step is proposed as a screening level effort to assess the potential human health risks associated with occupational and residential exposure to metals (and potentially agrichemicals pending the results of the effort described above in Section IV.B.1.a) in drinking water in the northwest region of Nicaragua.

#### **i. Data Collection**

We propose collecting a maximum of 200 drinking water samples from the same general locations as the surface soil samples (ie five categories of agricultural fields and five different residential areas as described above in Section IV.B.1.b.i).

In the agricultural fields, the exact sampling locations will be selected to characterize the primary sources of drinking water within each of the five categories of fields. The information about drinking water sources will be obtained from representatives of both NSEL and ASOCHIVIDA, the owners/operators of other agricultural fields, as well as from other retired workers who are not associated with ASOCHIVIDA. In the communities, drinking water samples will be obtained from the same residences where surface soil samples were collected. Accordingly, residences will be selected for sampling based on a consideration of both spatial variation (for surface soil) and drinking water source. A portable GPS will be used to record the exact coordinates of each sample location.

At each of the 200 sample locations, drinking water samples will be collected from the source directly into clean labeled sampling containers (generally plastic for metals and glass for organics, though certain agrichemicals may also require plastic containers). When measuring from a tap, first draw samples will be obtained. Water samples will be transported (cold packs) and stored (refrigerator) under cool conditions but not frozen. The aliquots in the plastic sample containers will be analyzed for metals via ICPMS and the aliquots in the glass containers will be stored for possible future analysis of agrichemicals. One field blank and one duplicate will be collected for each category of agricultural fields and in each community (total of 10 field blanks and 10 duplicates).

#### **ii. Data Analysis**

The descriptive analysis of the drinking water data will be conducted using summary statistics, graphical displays, and correlation coefficients. Shapiro-Wilks tests and graphical displays will be used to determine the nature of the distribution of the metals and agrichemicals in drinking water. For the purposes of statistical analyses, a log transformation of the data will be applied if necessary to produce an approximately normal distribution.

Method LODs will be estimated as three times the standard deviation of the field blanks. In cases where the mean field blank amounts are significantly different from zero ( $\alpha=0.05$ ), the corresponding data will be corrected by subtracting the mean field blank amounts from the sample amounts. Units for metals in drinking water will be reported as

mass per volume of water (ug/L or mg/L as appropriate). All statistical analyses will be conducted using SAS statistical software with statistical significance reported at the 0.05 level.

Levels of metals and agrichemicals in drinking water will be compared to USEPA Maximum Contaminant Levels (MCLs) and linear regression models will be used to determine whether contaminant levels are significantly different by microenvironment. For instance, are levels higher in drinking water collected from the agricultural fields compared to the residential areas? Are levels different among the different categories of agricultural fields or among the different residential communities? To the extent that CKD prevalence data are available for each of the residential communities, we will investigate whether contaminant levels by community are consistent with the observed differences in CKD prevalence.

An analysis of potential health risks associated with metals and agrichemicals in drinking water will be conducted in accordance with the USEPA's risk assessment guidance for human health evaluation activities (USEPA, 1989). Exposure concentrations in drinking water will be combined with exposure factors regarding contact rate, frequency, duration, and body weight to estimate contaminant intake (mg contaminant/kg bodyweight/day). Toxicity information (ie. RfDs, critical effects, uncertainty and modifying factors) will be obtained from USEPA's IRIS and combined with the intake estimates for the purpose of characterizing the potential risks associated with occupational and residential exposure to contaminants in drinking water. The cumulative risks associated with exposure to each contaminant in surface soil and drinking water will also be assessed.

#### **d. Food Samples**

The extent to which food may be contaminated with metals or aristolochic acid has not yet been explored in the northwestern region of Nicaragua. The potential for uptake of metals from soil varies by crop and growing conditions. Additionally, as was observed in the Balkan Endemic Nephropathy, there is potential for food to become contaminated with aristolochic acid.

As a first step toward evaluating the potential role of contaminated food, we propose to administer a short dietary survey to the 20 residents in each the five communities who participate in the surface soil and drinking water investigation (total of 100 surveys). The purpose of the survey will be to assess the types and sources of commonly consumed food so that food samples can be collected and analyzed for metals and aristolochic acid.

#### **i. Data Collection**

The short dietary survey will be administered during the collection of surface soil and drinking water samples so that food samples can be collected on a subsequent trip. The survey will be designed to identify the most commonly consumed food types (eg. rice,

beans, corn, flour tortillas, etc), the most common sources of those foods (eg. self-grown, market, farm stand, etc), as well as some limited food frequency information.

Since contaminant levels could vary by both type and source of food, we would ultimately design a market basket survey of non-meat food items that aims to capture this potential variability by focusing on the most common type/source combinations. We will also work with a botanist in Nicaragua to examine the fields where the food is grown to determine where *Aristolochia* species are present. Recognizing that there could be variation even within the same food type from the same source, we would also collect two samples of each type/source combination. Accordingly, if we collected two samples of the five most commonly consumed food types from the two most common sources in each of the five communities then we would collect a total of 100 food samples (2 samples x 5 types x 2 sources x 5 communities = 100 samples).

Each food sample will be collected from the same locations where residents obtain their food. The samples would be placed in plastic Ziploc bags, labeled, and frozen for shipping. One field blank and one duplicate will be collected for each type of food (total of 5 field blanks and 5 duplicates). For metals, the samples will be homogenized, extracted, and analyzed via ICP-MS. Though we are additionally interested in the possible analysis of aristolochic acid in food samples, the analysis of aristolochic acid is not a common analytical procedure with standardized protocols. We therefore must first evaluate the feasibility, logistics, and costs of analyzing for aristolochic acid in food prior to processing the samples for analysis of metals (in case the sample preparation procedures are different).

## **ii. Data Analysis**

The descriptive analysis of the contaminants in food will be conducted using summary statistics, graphical displays, and correlation coefficients. Shapiro-Wilks tests and graphical displays will be used to determine the nature of the distribution of the metals and aristolochic acid in food. For the purposes of statistical analyses, a log transformation of the data will be applied if necessary to produce an approximately normal distribution.

Method LODs will be estimated as three times the standard deviation of the field blanks. In cases where the mean field blank amounts are significantly different from zero ( $\alpha=0.05$ ), the corresponding data will be corrected by subtracting the mean field blank amounts from the sample amounts. Units for metals in food will be reported as mg/kg. All statistical analyses will be conducted using SAS statistical software with statistical significance reported at the 0.05 level.

Multiple linear regression models will be used to determine whether contaminant levels in food are significantly different by type, source, and/or community. To the extent that CKD prevalence data are available for each of the residential communities, we will investigate whether contaminant levels in food by community are consistent with the observed differences in CKD prevalence.

An analysis of potential health risks associated with metals food will be conducted in accordance with the USEPA's risk assessment guidance for human health evaluation activities (USEPA, 1989). The metals levels in each type of food will be combined with ingestion information from the food frequency survey and body weight to estimate contaminant intake (mg contaminant/kg bodyweight/day). Toxicity information (ie. RfDs, critical effects, uncertainty and modifying factors) will be obtained from USEPA's IRIS and combined with the intake estimates for the purpose of characterizing the potential risks associated with dietary metals. The cumulative risks associated with exposure to each contaminant in surface soil, drinking water, and food will also be assessed.

#### **e. Limitations**

The environmental sampling has been proposed and designed as a screening level effort given that there has been limited previous environmental sampling in the region. However, exposures to metals and agrichemicals likely occurred over many years and over a large geographic area, whereas our proposed investigation will focus on current conditions using samples collected from a relatively small area. Accordingly, there is the potential for findings from this activity to have a high impact if levels are elevated or if clear patterns are observed; however, the lack of elevated levels or the lack of clear patterns would need to be interpreted cautiously. Given the limited scope of the assessment, there could still be elevated levels that were missed because they are present in different areas or because they occurred at an earlier time and are no longer present.

## **2. Biological Sampling**

Partly based on the results of the environmental sampling, we will consider the benefits of analyzing biological samples (potentially including blood, urine, hair, nails, and bone x-rays) for metals, selected agrichemicals, and aristolochic acid. One potential source of samples is current workers at ISA, all of whom have routine blood and urine testing every year. A possible second source could be a random sample of the five communities in which the environmental sampling will be conducted..

### **a. Data Collection**

In each of the communities of La Isla and Candelaria, we would collect samples for analysis from 20 male sugarcane workers and 20 females who reside in the same homes (total of 80 samples from sugarcane communities). In each of the other three communities, we would select 10 males with a work history consistent with the primary occupation of the community as well as 10 females who reside in the same homes (total of 60 samples from non-sugarcane communities). Within each of the five communities, we will collect samples from a greater number of participants in order to determine eGFR so that we can ensure that the analytical samples reflect participants with sufficient variability of eGFR.

For metals, the blood samples will be shipped to an analytical laboratory, extracted, and analyzed via ICP-MS. We are additionally interested in the possible analysis of aristolochic acid in either blood or urine samples collected from this same group of subjects. However, given that the analysis of aristolochic acid is not a common analytical procedure with standardized protocols, we must first evaluate the feasibility, logistics, and costs of doing so. We will investigate these details for aristolochic acid prior to processing the samples for analysis of metals (in case the sample preparation procedures are different).

### **b. Data Analysis**

The descriptive analysis of the metals in blood will be conducted using summary statistics, graphical displays, and correlation coefficients. Shapiro-Wilks tests and graphical displays will be used to determine the nature of the distribution of the metals and agrichemicals in blood. For the purposes of statistical analyses, a log transformation of the data will be applied if necessary to produce an approximately normal distribution. Units for metals in blood will be reported as mass per volume of blood (ug/dL or modified as appropriate). All statistical analyses will be conducted using SAS statistical software with statistical significance reported at the 0.05 level.

Levels of cadmium, chromium, lead, and mercury in blood will be compared to the American Conference of Governmental Industrial Hygienists (ACGIH) Biological Exposure Indices (ACGIH, 2009). Multiple linear regression models will be used to determine whether contaminant levels are significantly different by work history or by sex. The assessment of community differences will be assessed separately for males

and females, since among males community will be highly correlated with industry, whereas among females the community differences are perhaps more likely to represent geographic variation (though work history will have to be taken into account for females as well).

Since serum creatinine levels and eGFR will be available for all subjects, we will assess metals in blood as potential determinants of kidney function. We will also use the eGFR data to classify subjects as CKD or non-CKD and assess metals in blood as potential determinants of CKD status using logistic regression models.

### **c. Limitations**

Similar to the environmental sampling, the biological sampling has been proposed and designed as a screening level effort. Biomarker levels integrate exposure across all exposure routes and pathways and could potentially yield information that would be missed if we relied on environmental samples alone. However, the half-life of metals in biological samples is shorter than in environmental media and we will be analyzing samples collected from a small subset of the population at a single point in time. Accordingly, there is the potential for findings from this activity to have a high impact if levels are elevated or if clear patterns are observed; however, the lack of elevated levels or the lack of clear patterns would need to be interpreted cautiously. Given the limited scope of the assessment, there could still be elevated levels that were missed because they are present in different subsets of the population or because they occurred at an earlier time but were no longer present in blood at the time samples were collected.

## **3. Work Observations**

The work observation study proposes to address two hypotheses: volume depletion and muscle damage. As described in section III.B.1, volume depletion is not typically considered a primary cause of CKD but rather is thought to represent an important risk factor for disease progression. In contrast, as described in section III.B.2, muscle damage and myoglobinuric acute renal failure may cause acute or chronic kidney failure. We also hypothesize that chronic recurrent muscle damage and myoglobinuria may be a cause of CKD even in the absence of overt acute kidney injury. The work observation will establish whether volume depletion and muscle damage occur in sugar cane workers, and if volume depletion and muscle damage are associated with renal damage as assessed through urine protein markers; this study has the potential to elucidate whether volume depletion and muscle damage contributes to the development and progression of CKD. However, we do not anticipate being able to definitively determine causality of these factors in regards to kidney disease based on these observations alone.

Major risk factors for volume depletion and muscle damage in this population include: ambient temperature and humidity, work effort, hydration status at the start of the work

day (which can be adversely affected by recent alcohol consumption), ability of the kidney to regulate perfusion at extremes of volume (which can be adversely affected by NSAIDS), and other medication use.

Accordingly, we will study workers in different environmental conditions and work environments. Subjects will represent three important occupational groups: (1) sugar cane harvesters, who are expected to be subject to the most extreme environment and most strenuous work conditions; (2) seed cutters, seeders, and weeders; and (3) a “control” group of sugar cane factory workers. We plan on studying 25 workers from each of the three groups, with repeated measurements from each worker over three workdays.

The cooperation of the union, current workers and ISA is essential and will need to be obtained prior to initiation of the project. In order to protect privacy of the workers and encourage participation, we would not share individual participant results with ISA and would offer a cash incentive for participation.

#### **a. Data Collection**

Related to this activity, we also propose to assess the industrial hygiene practices and health and safety program at ISA. The industrial hygiene assessment will include a review of work practices for each of the various tasks performed among field workers and factory workers, including work schedules, conditions, activities, personal protective equipment, etc. The review will necessarily focus on current practices but will attempt to assess how current practices may differ from historical practices by reviewing available documents and communicating with representatives of both NSEL and ASOCHIVIDA, representatives of unions, as well as with retired ISA workers who are not associated with ASOCHIVIDA.

For the work observation, the following data will be collected:

1. Prior to beginning work on the first day of the study, interviewers will obtain information on factors such as age, medication use (include over-the-counter and herbal preparations) in the previous seven days, recent alcohol intake, any known medical conditions, etc.
2. On each of the three days of observation:
  - a. Prior to beginning of work:
    - i. Physical examination: weight, height, sitting and standing blood pressure and heart rate, temperature
    - ii. Laboratory data:
      1. Stored blood: creatinine, creatine kinase, myoglobin
      2. Point of care urine: specific gravity, myoglobin (dipstick heme positive and if positive to be confirmed by specific assay), albuminuria

3. Stored urine: tubular proteinuria, quantification of myoglobinuria in participants with positive point of care tests
- b. During the work day
    - i. Wet Bulb Globe Temperature (WBGT): WBGT is a composite temperature used to estimate the effect of temperature, humidity, wind, and solar radiation on humans. WBGT readings will be obtained at multiple times throughout each work shift.
    - ii. Observations of work process: number of breaks, water consumption, duration of shift, intensity of work, etc.
  - c. After completion of work:
    - i. Hours of work, amount of cane harvested by work group (if cane harvester)
    - ii. Hydration interview: questionnaire assessing thirst using a Likert scale, access to fluids during work period, and whether each worker feels that they ingested adequate fluids during the day
    - iii. Physical examination: weight, height, sitting and standing blood pressure and heart rate, temperature
    - iv. Laboratory data:
      1. Stored blood: creatinine, creatine kinase, myoglobin
      2. Point of care urine: specific gravity, myoglobin (dipstick heme positive and if positive to be confirmed by specific assay), albuminuria
      3. Stored urine: tubular proteinuria, quantification of myoglobinuria in participants with positive point of care tests

As with the review of industrial hygiene practices, we will attempt to assess the extent to which conditions during the monitoring week reflect historical conditions with respect to factors such as work intensity, work duration, number and length of breaks, and water consumption. For this reason, we will ask appropriate representatives from NSEL, ASOCHIVIDA, unions, and other retired workers (who aren't associated with ASOCHIVIDA) to accompany the field team during the monitoring effort and provide some feedback regarding the representativeness of the observed days.

### **b. Data Analysis**

The following will be the focus of comparison between cane cutters and factory workers:

1. Estimates of volume status: weight change, change in systolic and mean arterial pressure during the work day (both sitting and standing), change in temperature, change in serum creatinine, urine specific gravity at end of work day, differences in hydration interview
2. Estimates of muscle damage: change in serum creatine kinase and serum myoglobin, presence of dipstick myoglobin (with positive confirmation by direct assay)

If we find that volume depletion and muscle damage are occurring, logistic regression analysis will be performed to look for possible risk factors, including age, environmental temperature, work effort, adequacy of hydration, thirst, and recent medication use. WBGT data will be compared with ACGIH threshold limit values (TLVs) for working in hot environments (ACGIH, 2009).

**c. Timeline**

We estimate that the three sets of activities described above (environmental sampling, biological sampling, and work observation) can be completed within 12 months from the time we are authorized to proceed. The three month range for each set of tasks is intended to represent the duration of the task as much as it is to reflect the uncertainty. For instance, prior to collecting or analyzing any samples it is necessary for us to finalize the protocol and obtain approval from our Institutional Review Board (IRB). However, we recognize that finalizing the protocols will involve communication with representatives of both NSEL and ASOCHIVIDA, and experience tells us that IRB approval can take 4 to 6 weeks (from initial submission to approval, including inevitable revision). As another example, once samples are sent to an analytical laboratory, the turnaround time can vary widely depending on their availability. The work observation study could be done early in the project and could be done over two weeks either consecutively or interrupted. Logistical issues will include identifying and hiring personnel who can be trained or are familiar with taking vital signs and performing phlebotomy. We would also need to enlist the cooperation of NSEL, the union and current workers in order to recruit appropriate subjects. Accordingly, the following is our best estimate of the time it will take to complete the tasks described in Sections 4.B.1 through 4.B.3.

Activity Description	Months											
	1	2	3	4	5	6	7	8	9	10	11	12
Assessment of agrichemicals	X	X	X									
Investigate options for aristolochic acid analysis	X	X	X									
Develop dietary questionnaire, prepare protocol, and obtain IRB approval	X	X	X									
Collect and analyze biological samples				X	X	X	X	X	X			
Administer dietary questionnaire & collect and analyze soil and drinking water samples				X	X	X						
Conduct work observations and analyze samples				X	X	X						
Collect and analyze food samples						X	X	X				
Data analysis and reporting							X	X	X	X	X	X

#### **d. Limitations**

As discussed, it will be important to ensure that the working conditions during the observation study are representative of typical current and historical work practices, in terms of environmental conditions and work intensity. Similarly, the workers studied would also need to be representative of those at risk, specifically their physical conditioning and work effort should be within the range of a typical worker. To ensure to the extent possible that these requirements are met will require cooperation from a number of stakeholders, including representatives from NSEL, ASOCHIVIDA, unions, and other retired workers (who aren't associated with ASOCHIVIDA). It might prove useful to use employment records to develop a profile of a typical worker in terms of height, weight, age, and work effort. Even with these precautions, it is possible that because the workers are being observed their behavior, particularly in terms of fluid repletion, may be better than usual practice. As such, we expect that data may represent practices somewhat better than typical. Therefore, the absence of signs of volume depletion or muscle damage will not absolutely exclude these factors as important in the development of CKD in the population at risk.

The markers chosen for muscle damage, creatine kinase and myoglobin, are relatively sensitive but may not detect all instances of subclinical muscle damage. Nevertheless, as indicated in a previous study of exercise in normal volunteers (Clarkson, 2006) these markers were able to detect a remarkably high incidence of muscle damage. The use of markers of tubular proteinuria to detect kidney damage should be considered exploratory, since there are no preliminary data establishing their utility in patients with volume depletion or myoglobinuria. The absence of positive findings would not definitively exclude the possibility of subclinical renal damage, but alternative measures such as inulin clearance or histologic examination are not practical.

### **4. Cohort Study of ISA Employees**

We recommend assembling data from past and present NSEL workers in order to determine the association between occupational characteristics and the occurrence of CKD. This cohort will be assembled from employment and medical records; workers will be categorized according to several exposure definitions, and analyses will determine the incidence of abnormal test results, symptoms, disease, and death. We have had two opportunities to discuss the content and availability of the records at ISA with company representatives, as well as to view the records and storage systems. Our understanding of the exact nature and condition of the records is still evolving—and one of the first proposed activities is to conduct a feasibility/pilot study to gather and critically review existing occupational and medical records and determine the practicality of accomplishing the proposed study. Based on current information, we believe that the availability, completeness, and quality of data, while not without problems, will allow us to successfully conduct a study.

#### **a. Background**

As noted previously, NSEL is the largest of four sugar companies in Nicaragua;

approximately 5,500 persons work at the ISA each year, the great majority of whom are primarily involved in sugar cane cultivation, harvesting, processing, and manufacturing on approximately 40,000 hectares of land. The cane is harvested and planted during the months of November–April. People who work at ISA are divided into three categories: permanent employees, temporary employees, and contractors. The size of the permanent workforce is about 600. Approximately 5000 temporary employees and contractors—including about 1500 cane cutters—are hired for the harvest months (November-May), and 800 temporary workers work the remaining six months.

#### **b. Employment records**

Employment records provide the basis for worker identification. According to information provided by company representatives, these records have been kept since the 1960s. In recent years, records have been computerized, with prior records available on paper.

We plan to use employment and payment records to construct a detailed employment history for individual workers. Due to the seasonal nature of the ISA's work, we anticipate linking annual work records for each worker (through their social security number) for each year worked and then compiling his or her complete work history. If necessary, annual screening records, described in the outcomes section, may be used to supplement data missing from employment records.

Employment records are expected to contain job title and dates of employment, allowing us to determine the amount of time spent in each job and characterize workers according to whether they ever, usually, and currently work in a particular job title; to distinguish full-time vs. seasonal employment; and to determine duration of employment as well as ages worked. We plan to group workers into the following six categories:

- Cane cutters
- Seed cutters, seeders, and weeders
- Pesticide applicators
- Factory workers
- Office/administrative
- Other miscellaneous

During different time periods, workers may have been more or less likely to carry out tasks that fall under more than one job category at different times of the year. In order to better categorize job tasks, we will carry out a careful review of records as well as obtain information from company representatives (as part of the industrial hygiene activities described as part of the work observation activities; see Section IV.B.3), and develop and administer a structured questionnaire to current and former workers.

#### **c. Exposure categorization**

In Section III, we suggested three main hypotheses regarding a possible occupational etiology for CKD in this setting: exposure to agrichemicals, volume depletion (either independent of or in conjunction with other exposures), and muscle damage (again

either independent of or in conjunction with other exposures). The latter two hypotheses are closely related and are unlikely to be distinguishable based on data abstracted from records, but information gleaned from work observation should provide a basis for determining the interplay between these two mechanisms. Therefore, for purposes of exposure categorization we have treated them as a single hypothesis.

*Agrichemicals:* We plan to use job history data to estimate exposure to agrichemicals. Job titles can be categorized according to potential for exposure to agrichemicals determined to have nephrotoxic potential based on toxicological review (see Section IV.B.1.a) based on information from company representatives, current and former workers, company records, our environmental sampling, and literature describing typical exposure patterns in sugar cane cultivation, including frequency and season of application. This information can be used to construct an index of exposure based on job title, duration, and calendar year (in order to capture changes in pesticides and practices employed over time).

*Volume depletion/muscle damage:* We also plan to use job titles to classify work intensity based on results from the work observation (see Section IV.B.3). In addition, because cane cutters are paid on a piecework basis, company payment records will be used to approximate the amount of tonnage cut, which will in turn be used to construct a measure of work intensity among cane cutters. There can be a twofold difference between the amount of tonnage cut by different workers (personal communication, Dr. Carlos Alonso Medrano, 6/09). We recognize that this is an imperfect measure, both because disparities in tonnage harvested may be due to differences in skill and efficiency in addition to effort expended and because the affinity group, which typically numbers about four workers, is the smallest unit for which tonnage can be calculated and payment determined. However, we expect that there remains a reasonable correlation between amount of effort expended and amount of sugar cane harvested.

*Covariates:* We also plan to collect covariate information on past work history; location of residence; body mass index; co-morbidities such as diabetes and hypertension; family history of kidney disease; and history of cigarette smoking, alcoholic beverage consumption, and use of nephrotoxic medications. Covariate information should be primarily available through medical chart review and pre-employment screening records, described in the section on Outcome Assessment.

#### **d. Study design and follow-up**

Eligibility criteria will be decided after we establish the earliest date that CKD occurrence can be determined. Annual screening for creatinine began in 1996. Therefore this is the start of follow-up for the cohort study with hypotheses involving creatinine level as the main outcome of interest. Follow-up will continue through December 31, 2010.

#### **e. Outcome assessment**

We will review available medical records (from ISA and non-ISA sources) to obtain information on the occurrence of medical outcomes across the spectrum of kidney

disease from abnormal kidney function test results to symptoms, disease incidence, and mortality. The sources of medical records are:

- i. **ISA Hospital.** Throughout the entire period, the ISA has had a hospital on its grounds that has provided both inpatient and outpatient care free of charge for current employees and their families and for retirees. Prior to 2003, most employees lived on the ISA grounds and received virtually all their care at the ISA hospital. Almost all medical records are on paper, and are stored on site. They are filed according to social security number, and it appears that individual records can be located without much difficulty. However, this will be tested empirically during the feasibility/pilot phase of the study. These records contain information on diagnosis of CKD; yearly routine creatinine testing, urinalysis; and physical exam on all permanent employees, other conditions, and prescribed medications. These records also contain some information on past work history that may prove useful for exposure characterization.
  
- ii. **Annual physical exam for contracted employees (2003 to present).** Prior to 2003, all persons working at ISA were employees of NSEL. Beginning in 2003, workers who harvested the cane were hired on an annual basis through subcontractors. The yearly physical exam results for this group have been obtained and stored separately. The exam gathers information on a variety of physiological parameters, including serum creatinine level, urinalysis, and blood pressure. In addition to the exam, a baseline questionnaire is administered at time of hiring that obtains data on a number of factors relevant to the investigation (Table 14). More recent records have been computerized, and company plans are to computerize the earlier records as well, which may be completed in time for this study.

**Table 14. Contents of questionnaires administered to contract employees at time of pre-hiring screening**

<b>2004 Questionnaire</b>	<b>2008 Questionnaire</b>
Current Address/Municipality	<b>X</b>
Municipality lived in during childhood	<b>X</b>
Current job	<b>X</b>
Employment history	Employment history
Employment history with sugar refineries	Employment history with sugar refineries
Alcohol consumption	Alcohol consumption
Illegal drug use	Illegal drug use
Smoking history	Smoking history
Daily water consumption	<b>X</b>
NSAID use	NSAID use
Injected medication history	Injected medication history
Pesticide exposure	Pesticide exposure

Family history of kidney failure	Family history of kidney failure
Personal history of hypertension	Personal history of hypertension
Personal history of Diabetes	Personal history of Diabetes
Personal history of repeated urinary infection	Personal history of repeated urinary infection
Personal history of Malaria	Personal history of Malaria
Personal history of Leptospirosis	Personal history of Leptospirosis

- iii. **Local health centers and regional hospitals (2003 to present).** Some workers who can no longer work because of high creatinine tests receive follow-up care at the local health center (Centro de Salud) in Chichigalpa, which has a dedicated CKD unit and where they also fill out the paperwork to apply for government benefits available to them as a result of no longer being able to work. A smaller number use other local health centers or the regional hospital (Hospital España) located in the town of Chinandega. We have met with representatives from both the Chichigalpa health center and regional hospital and have been informed that we could have access to medical records.

Access to these medical sources allows us to compile a wide range of medical information that may serve as endpoints of interest (e.g., serum creatinine levels) descriptors of disease (e.g., proteinuria and ultrasound findings), or confounders that we can control for (e.g., blood pressure). For example, the annual exams provide an ongoing measure of kidney function, assessed repeatedly for individuals. In addition, we can determine onset (incidence) of CKD or mortality, and dates that these occurred.

The primary outcome for the study will be a serum creatinine measurement of  $\geq 1.2$  mg/dl, because that is the level above which workers are not permitted to work at the ISA. However, because a single creatinine of 1.2 mg/dl is sensitive but not specific measure of CKD, we will also follow individuals up for a diagnosis of CKD in the medical record and additional tests consistent with a diagnosis of CKD. We will develop criteria to classify cases as definite, probable, and possible based on the available information.

According to company representatives, workers who are let go because of high serum creatinine levels sometimes return the following year using a different name and social security number. This problem would be more likely to occur in later years (after 2002), and there does not appear to be any way to identify these individuals. However, it is not perceived to be a widespread practice and is further minimized by the fact that it should impact only those individuals whose creatinine was originally  $\geq 1.2$  mg/dl and then decreased below the proscribed level in the intervening time period.

**f. Sample size**

Approximately 20,000 workers have been employed by ISA during the period of time

covered by this study. Because most employment and medical records are not computerized, we do not believe that it is feasible to review the records of all workers employed during the follow-up period. An estimated 2,000-3,000 records of workers who have developed CKD appear to be available at ISA. A review of 4,000 randomly selected records would result in an expected 500-600 cases of CKD. The final sample size and sampling strategy will be informed by the results of the feasibility/pilot study.

#### **g. Data analysis**

Social security number should be available on both employment and medical records and we plan to use it as the primary variable for linking records, supplemented by company ID number, full name, date of birth, and gender. A subject's follow-up begins at the start of follow-up (January 1, 1995) if they started working prior to that date ("prevalent hire") or at the time of hire if they are hired during the follow-up period ("incident hire"). Follow-up ends at the earliest of the following: onset of poor kidney function (as measured by serum creatinine level), mortality, date of last known medical visit, or end of follow-up (December 31, 2010). Subjects will be excluded if they had prevalent kidney disease at the start of their follow-up time.

The baseline date will be unique for each subject depending on when follow-up starts. The timeline is then time in the study. The data will be analyzed using Cox proportional hazard regression to compare the incidence of each kidney outcome described above according to employment characteristics. We will consider several assumptions for the induction and latent period in the crude and adjusted analyses. Potential confounders for the latter will include gender; age (at baseline); BMI; family history of kidney disease; personal history of diabetes, hypertension, cigarette smoking, and use of nephrotoxic medications; and residential location(s).

Because many subjects will have begun working prior to follow-up starting in 1995 the study will include prevalent hires (began working prior to 1995) as well as incident hires (began working during study follow-up, 1995 through 2009). Prevalent hires represent a group that was subjected to left truncation, where subjects who were hired prior to the start of follow-up and are still working when follow-up begins may not be representative of all subjects who worked during that time (e.g., the healthier subjects remain at work). We decided it was important to include prevalent hires both because exposure conditions have changed over time and because restricting to persons hired after 1995 would exclude most ASOCHIVIDA members, for whom the study results are most relevant based on the objectives of the Dialogue. Further, prevalent hires are not an inherent problem if they are addressed in the analysis. We will address this issue by using a time-dependent Cox model. Further, we will examine exposures separately that occur during follow-up (time-varying) and exposure status before the start of follow-up (constant).

Finally, in additional analyses, we will describe the characteristics of kidney disease wherever possible. This will include abstracting all data on urinalyses (specifically the presence and qualitative amount of proteinuria and hematuria on urine dipsticks) and all ultrasound data (particularly descriptions of kidney size and echogenicity) on members

of the cohort. Given the suspected prevalence of kidney disease in this region of Nicaragua, both the cross-sectional relationship and the temporal relationship between proteinuria and estimated GFR could be helpful to narrow the differential causes of CKD in this region. Further information on this specific portion of the project is provided in Section 5 (Medical Record Review), and we will strive to coordinate these efforts to maximize efficiency.

#### **h. Project Timeline**

The study activities, which will require a minimum of 19 months, will be divided into two phases: a feasibility/pilot phase lasting five months and a main study phase lasting 14 months. We will first conduct a feasibility study that will include a detailed and critical review of existing occupational records, occupational exposure assessments and records, employment records, medical care facilities and available medical records. Understanding the availability and quality of these records will be an essential component of refining the proposed cohort study. During this phase we will also conduct a pilot study, which will be based on 50 occupational and 50 linked medical records, to determine the most efficient and feasible manner for conducting the main study. In particular, we will assess the organization of occupational and medical records and will pre-test record review and linkage procedures and data collection forms during this period. We will then prepare a preliminary report summarizing the availability, completeness, and quality of records and the feasibility of their use for a cohort study. The report will be reviewed by the Scientific Advisory Board. The timeline for the entire study is given below.

**Months 1-4:** Conduct feasibility study, including collection and critical review of existing occupational records, occupational exposure assessments, employment records, medical care facility record systems, and available medical records. Develop procedures and code book, hire local staff for pilot and main study, conduct pilot study of 50 linked occupational and medical records.

**Month 5:** Evaluate results of feasibility/pilot study, revise study design as indicated by feasibility study, develop procedures and code book.

**Months 6-15:** Review records, computerize and clean data.

**Months 16-19:** Conduct data analysis, write report.

We currently estimate that it will take approximately 6,000 hours to review 4,000 records, particularly given that the review of medical records also is being conducted to address additional hypotheses unrelated to occupational exposure (see Section IV.B.5). We anticipate that this task will require 8 individuals at 750 hours/person (20 hours/week for 37.5 weeks) and a senior epidemiologist based in Nicaragua to oversee their work. The considerable logistics issues would be handled by the Nicaraguan-based research assistant. Double data entry would be done in the U.S. (or Nicaragua if acceptable), and a programmer's time would be required for data analysis. . These estimates may require revision if indicated by the feasibility and pilot activities.

## **i. Limitations**

We have assumed that existing company, employment, environmental sampling, medical care facility and individual medical records are sufficiently detailed and valid to be able to conduct the above described study. While it is likely that basic information such as job title and dates of employment will be available, it is less clear whether more detailed data will be obtainable. For example, it is unclear if historical records on environmental sampling will be sufficient to construct a valid index of exposure to specific agrichemicals. Thus, depending on the results of the feasibility/pilot study, it is likely that proposed study design and protocol will be refined. Any important changes in scope will be submitted for review to the Scientific Advisory Board prior to implementation.

## **5. Medical Record Review**

Medical chart review represents a potentially high yield source of data to help elucidate the nature of and medical correlates of kidney disease among former workers at the ISA. In recent years, reflecting a perceived high prevalence of kidney disease among workers, the ISA had instituted a screening program for kidney disease. Potentially available data involve multiple sources, including the ISA data which would serve as a baseline:

- i. ISA Employment Medical Charts: Charts containing data on basic demographics and medical history obtained from the individual worker, work history within the ISA, blood pressure and other physical examination items, serum creatinine and urine dipstick results (personal communication with Felix Zelaya, 6/09);
- ii. ISA Medical Clinic Charts: Charts from the clinic at the ISA, which, for individuals diagnosed with kidney disease, will often contain extensive longitudinal data including renal ultrasounds. These medical charts would also include records of medications dispensed by the clinic. It is estimated that there are 2000-3000 charts of individuals with kidney disease; of note, individuals who were not continuously employed by the ISA may not have received chronic medical care at this facility (personal communication with Drs. Antonio Marin and Mauricio Jarquin, 6/09);
- iii. Julio Duran Zamora Health Center Charts: Records from the local medical clinic in Chichigalpa (Julio Duran Zamora Health Center), the site of a busy nephrologist clinic where local residents who cannot receive medical care at the ISA are treated until the end stage of kidney disease (personal communication with Edwin Reyes, 6/09); and
- iv. Hospital España Charts: Records from the Hospital España in Chinandega where individuals with kidney failure would seek higher level medical care.

Screening for kidney disease, consisting of serum creatinine measurement and urine dipstick analysis, is performed at least annually among temporary and contracted ISA workers. For sugar cane cutters, testing occurs at the start of the season, in the middle of the season and at the completion of the cutting season (three times in a 6 month period), although, per report of the physician staff at the ISA (personal communication with Felix Zelaya, 6/09), the final test of the year often fails to occur for seasonal workers. Workers are assigned a unique identifier for testing, and on the medical chart a small picture is included.

Performed systematically, chart review has the potential to help determine the cause of kidney disease, specifically discriminating between risk factors for and presence of glomerular disease versus manifestations more consistent with tubulointerstitial disease.

i. Tubulointerstitial disease versus glomerular disease

In order to help differentiate between tubulointerstitial and glomerular kidney disease, we will abstract serial creatinine and urine dipstick results. In the setting of rising serum creatinine and minimal proteinuria, particularly with kidneys that on later ultrasound evaluation appear uniformly small, it is likely that the cause of kidney disease is tubulointerstitial

ii. Medications as an etiology for tubulointerstitial disease

In order to explore medications as a potential etiology of kidney disease, we will abstract medication prescriptions for workers who received medical care at the ISA. Strengths include potential ability to evaluate temporal relationships between medication prescription and evidence of kidney disease. Limitations include the distinct possibility that workers may have obtained medications from other sources

iii. Glomerular Disease, Diabetes and Hypertension

We will abstract medical history and physical exam findings from these records, specifically evaluating the temporal relationship between blood pressure and serum creatinine levels as well as attempting to identify a history of diabetes or other well-known causes of kidney disease. In the course of this process, as stated above, we will record the measured dipstick proteinuria as, in the absence of significant proteinuria (>1+) preceding rising serum creatinine, it is unlikely that the cause of CKD is a glomerular process

iv. Kidney stones

Because nephrolithiasis is highly prevalent in regions of high heat and humidity and recurrent nephrolithiasis has the potential to cause CKD, we will review charts of persons with and without CKD to determine the prevalence of kidney stones. This is likely to be of low yield.

Chart review will be conducted as part of the data collection for the cohort study, and therefore will not require additional personnel or other costs.

## 6. Urinary protein determination in adolescents

The purpose of this study is to determine the prevalence of CKD prior to subjects entering the work place. This reflects the hypothesis that CKD may be prevalent in the general population but that sugar cane cutters have a more rapid progression to later stages of kidney disease due to recurrent volume depletion, myoglobinuria, and potentially other exposures or lifestyle practices. As previously discussed, markers of kidney damage may include increases in serum creatinine, proteinuria, abnormalities on renal imaging, or pathologic changes on renal biopsy. Serum creatinine is an insensitive marker of early kidney disease, as, by the time there is a significant rise in serum creatinine, tremendous renal reserve has already been lost. Additionally, serum creatinine assessment would require a blood draw. Renal imaging is also insensitive and is a relatively expensive screening test. Kidney biopsy is expensive, invasive and poses risks that include death and kidney loss; furthermore, kidney biopsy is unlikely to lead to the diagnosis of a treatable condition in the screened population.

Accordingly, we are focusing on urine proteomics to identify early kidney disease. As previously discussed, it is likely that CKD in Nicaragua is a tubulointerstitial condition. As such, tubular proteinuria would be one of the earliest detectable manifestations of disease. Tubular proteinuria describes low molecular weight proteins that are normally filtered by the glomerulus and then metabolized by the proximal tubule cells; these proteins are found in the urine when tubulointerstitial disease interferes with proximal tubular reabsorption and metabolism of these proteins. Beta-2 microglobulin, which appears in the urine through this mechanism, has been used to diagnose tubulointerstitial disease in children (Portman RJ, 1986). In addition, tubular epithelial cell proteins may be released into the urine from damaged tubular cells. A number of such proteins have been identified in the urine including  $\alpha$ -glucosidase and N-acetyl- $\beta$ -D-glucosaminidase (NAG) (Barratt J, 2007).

The work proposed consists of collecting urine samples from adolescents aged 12-16 years. Our hypothesis is that if there is epidemic chronic tubulointerstitial disease due to heavy metals, aristolochic acid, or other nephrotoxins, or from hereditary tubulointerstitial nephritis, early indicators of kidney damage, such as tubular proteinuria, will be manifest. With a prevalence of overt kidney damage as determined by low eGFR of 5-15%, one might expect more sensitive markers such as tubular proteinuria to be present in an even higher percentage.

We will select subjects for this study to include children of cane workers with known CKD, nieces and nephews of affected workers whose parents are not affected, and children whose parents have never worked in sugar cane. Approximately 100 children will be studied with equal numbers of males and females. Random, clean catch urines will be collected with appropriate preservatives and storage. Testing will include:

1. Dipstick for proteinuria/albuminuria
2. Urine albumin to creatinine ratio, a quantifiable measure of early glomerular proteinuria.
3.  $\beta$ 2 –microglobulinuria, retinol binding protein, and n-acetyl- $\beta$ -D-glucosaminidase, all of which are markers of tubular proteinuria.

This study could be begun early in the project but would take several months to identify and recruit potential subjects. Personnel to collect samples will need to be hired. We will also need to obtain cooperation and consent of present and past workers, and parents to identify potential subjects.

The yield of this study is clearly dependant on tubulointerstitial disease remaining the leading diagnostic possibility as a cause of CKD in this region. The advantages of sampling older children is that there would likely be adequate time for a local factor to cause at least an early manifestation of kidney damage but most have not yet been exposed to the taxing work environment that may offer additional factors leading to progression of CKD.

## **7. Post-mortem renal biopsy**

Although renal biopsy is often useful in glomerular diseases for defining etiologies, for chronic tubulointerstitial disease biopsies often fail to identify the cause of CKD. In part this reflects the presence of scarring and fibrosis, a non-specific finding in most late stage tubulointerstitial processes. Early in the disease course, particularly in conditions with acute onset, biopsy may be able to distinguish causes such as drug reactions or nephrotoxins; however, pathologic findings may only be specific for the first few days or weeks after the insult.

There is potential utility in obtaining kidney biopsies early in the course of the disease or in people without clinical manifestations to determine if early pathologic abnormalities are present. However, since renal biopsy is associated with some risk, including death, and it is unlikely that a biopsy would alter the therapy for the renal disease, there are ethical concerns regarding performance of biopsies. One possible solution would be to obtain post-mortem renal biopsies in people dying from acute trauma, such as motor vehicle accidents. We understand that deaths from motorcycle accidents in people not wearing helmets are common and these victims are often young males, the group which is at risk for CKD. At least two groups have informed us of their plans to conduct biopsies on living persons with early kidney disease. Although we would like to discuss our ethical concerns with them, if they do choose to proceed with permission from ethics boards in Nicaragua, we would take advantage of the information they generated, and would likely not continue with this activity.

Assuming we do proceed, we would propose initially doing 10 post-mortem renal biopsies, processing the tissue only for light microscopy. Depending on the initial results we may want to obtain additional biopsies and include processing for immunofluorescence and electron microscopy. Additional evaluation could examine for aristolochic acid DNA adducts. Logistical barriers for post-mortem biopsy are formidable, with substantial cultural barriers against allowing autopsies with some of these beliefs potentially carrying over to post-mortem biopsies. We would have to enlist the cooperation of personnel at the local hospitals where accident victims are taken to

identify patients and either contact study personnel to obtain the consent of families or to obtain the consent themselves. Finally, we would have to enlist the cooperation of a radiologist to perform the biopsies under ultrasound guidance.

#### **a. Timeline**

This study could be begun early in the project but would take several months to identify and enlist the cooperation of appropriate hospital personnel and perhaps provide some education to the community. Other organizations have informed us of their plans to conduct biopsies on living persons with early kidney disease. Although we would like to discuss our ethical concerns with them, if they do choose to proceed with permission from ethics boards in Nicaragua, we would take advantage of the information they generated, and would likely not proceed with this activity.

#### **b. Limitations**

The logistical barriers, including cultural taboos, informed consent, recruiting hospital personnel to perform the biopsies, and tissue processing make this effort a formidable undertaking. Optimally we would require creatinine measurements on potential subjects to exclude significant kidney disease, and these measurements may not be available on accident victims. In the absence of knowing the specific cause of the epidemic CKD this effort is also largely exploratory. There are some potential toxins that can be specifically identified in the kidney, such as aristolochic acid, but for others the histologic findings may be non-specific. However, if done sufficiently early in the disease process, it could tell us whether we are dealing with a glomerular or tubulointerstitial disease. However, because the population on which we can perform biopsies may not be representative of the population at risk, any conclusions would be tentative. As discussed, access to the results of a planned broader kidney biopsy study would enhance our efforts

### **8. Interviews**

We propose to conduct two types of interviews for the purposes of *a)* refining our data collection plans for what we consider to be immediate, high priority hypotheses and, *b)* exploring hypotheses that we could not otherwise address using qualitative methods at relatively low cost and potentially high return. The first set of interviews that we propose, key informant interviews, will be conducted at the earliest time possible in our research and will not require informed consent. Key informants will *inform* our research from the outset; they are not research subjects or participants. Our later interviews, the qualitative one-on-one interviews with physicians and care providers, will require informed consent. Research participants will be selected based on considerations related to the likelihood of the generalizability of our findings, and the transcripts of these interviews will be analyzed using standard methods for qualitative data analysis.

#### **a. Key Informant Interviews**

In developing our hypotheses regarding CKD, we have identified several exposures that have the potential to cause CKD but we are presently lacking information that would

enable us to develop and test hypotheses regarding these exposures and CKD in Nicaragua. For these exposures, lija consumption, the use of herbal medicines, and occupational exposures, more information is needed before a study plan can be completely developed. In order to obtain this information, we propose to interview 14 - 19 individuals, or "key informants," who can provide us with specialized information that helps us refine our research during the early stages of investigation. Key informant interviews, where researchers question a few individuals based on their expertise, are a commonly used research tool in the social sciences. They provide information that may be too difficult or time-consuming to uncover through more structured data gathering techniques such as surveys or semi-structured one-on-one interviews. The main criteria for choosing key informants are the amount of knowledge the individual has about the topic of research, and his or her willingness to cooperate with the research team (Blee and Taylor, 2002).

We propose to conduct key informant interviews with people who or are knowledgeable about the following exposures so that we may begin to fill-in some of the gaps in our present understanding:

#### **i. Lija**

As described in Section III.B.11, lija is a form of rum produced in concentrated form at a commercial distillery and then diluted (with the possibility of introduction of toxic substances) after it is shipped to distributors and retailers. Lija is primarily consumed by persons who cannot afford bottled rum. Lija is a troubling hypothesis because it has been consistently associated, often very strongly, with CKD in case-control studies (see Section II.B.4.c and Table 10i). Although confounding by socioeconomic status is certainly a potential explanation, even in three studies that appear to be conducted among persons of the same socioeconomic characteristics (i.e., sugar cane employees), odds ratios for consumption of lija have ranged from 6-11 (Zelaya, 2001; Alonso, 2002; Callejas, 2003). Importantly, the pattern of epidemiologic results for lija in particular differs from alcohol in general. These results are both hard to accept and difficult to dismiss. They are difficult to study by questionnaire because they are subject to recall bias (though perhaps in unpredictable ways), and current samples cannot necessarily be used to test for historical exposure, because the introduction of toxic adulterants would have very likely occurred on a sporadic basis. In addition, despite numerous discussions, we still do not have a clear picture of the system for distribution and sale of lija nor its consumption by different groups in the population.

We propose to interview 4 – 6 individuals who have knowledge regarding historical and current use of lija, including MINSA, law enforcement officials, physicians, cooperative distributors and retailers.

#### **ii. Herbal medicines**

As noted in Section III.B.9., herbal ("natural") medicines appear to be used with some frequency by many individuals. In other settings, treatments of this type have led to disease outbreaks, including CKD (Section II.A.). Although we are aware of some of the specific medicines used, we expect there are additional medicines or herbal remedies in

Nicaragua of which we are not aware. In addition, we know little about their geographic distribution, usage pattern (e.g., differences by sex), contraindications, and toxic properties.

We propose to interview 5 – 6 individuals who have knowledge regarding the historical and current use of herbal medicines and their constituents, including botanists, toxicologists, cultural anthropologists, physicians, and lay/traditional healers local to the region.

### **iii. Occupational exposures**

A number of our hypotheses require a complete understanding of the daily and seasonal patterns of workers in various jobs at ISA. For example, to conduct environmental sampling in areas where people work, we need to understand the times of year certain activities are conducted and the location of ISA workers during these times. This is also true of the proposed work observation and cohort study of ISA employees. We have heard from NSEL, for example, that some workers do multiple jobs in a season, while others may only work on a single job at various times during the year.

We propose to interview 5 - 7 individuals who have knowledge regarding the practices of NSEL workers including knowledge of job tasks and responsibilities, location of workers in a variety of job tasks at ISA, season and duration of tasks, and historical and current agricultural use of land parcels in and around NSEL and the pesticides usage on these areas. These interviews will include NSEL /ISA personnel, and current and former workers.

Each key informant interview will last approximately 30 minutes and contain a mix of closed and open-ended questions. Information gathered during the interviews will inform the generation of hypotheses regarding the specific exposures, and subsequent research activities. A written report summarizing the responses for each hypothesis above and our proposed next steps will also be shared with interested parties (i.e., NSEL and ASOCHIVIDA).

### **b. Qualitative one-on-one interviews**

The term, qualitative research, frequently refers to a variety of approaches and techniques including one-on-one interviews (Snape and Spencer, 2003). Many qualitative research methods rely on the technique of asking open-ended questions to yield in-depth responses about people's attitudes, beliefs, experiences, opinions, perceptions, and knowledge (Patton, 2002). Qualitative methods also facilitate data collection on individual, contextual, and cultural factors that contribute to complex diseases (Foster and Sharp, 2005). In this instance the hypotheses of medications and urinary tract infections will be best pursued using qualitative interviews designed to obtain information on provider beliefs and opinions regarding the diagnosis and treatment of UTI that may lead to the practice of prescribing medications. We will ask about the decision-making process and a variety of considerations that may result in a diagnosis. Individual, one-on-one (as opposed to group) interviews allow for people to

more freely express themselves without the constraints of peers or a particular social or professional setting. Respondents will be asked to sign consent forms, and told that their particular responses will not be shared with our project collaborators or the general public.

The one-on-one qualitative interviews we conduct to address medications prescribed to patients will differ from the key informant interviews for the following reasons: 1) care will be taken to establish a sample of respondents that generally reflects the types of care providers in the region, 2) confidentiality of responses will be assured to all respondents, 3) interviews will be semi-structured using a pre-designed set of open-ended questions to be asked of all respondents, and 4) data collected will be analyzed using the qualitative analysis software, NVIVO.

We propose to explore these hypotheses using open-ended questions because this style of investigation enables the researcher to hear and make sense of a response from someone being interviewed without predetermining their points of view by response categories fixed by the researcher ahead of time, as in quantitative survey methodology. Questions likely to elicit a “yes” or “no” response, are not open-ended, nor are questions that lead people to a type of response, for example, not stressful, somewhat stressful, very stressful. Conventional survey methods identify the possibilities ahead of time and do not allow for additional, surprising, or multifaceted responses.

#### **i. Medications.**

As described in Section III.B.9, a number of conventional medications are associated with CKD, including common non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, naprosyn and diclofenac, all of which are used widely in Nicaragua. However, kidney failure associated exclusively with NSAIDs is unusual; rather NSAIDs are more often a cause of acute renal failure in the setting of severe volume depletion or other nephrotoxins. Such nephrotoxicants include aminoglycosides, broad-spectrum antibiotics requiring either intravenous or intramuscular administration. Aminoglycosides are well known to cause kidney failure, with risk factors including pre-existing kidney disease, concomitant nephrotoxic medications, advanced age, and dehydration/volume depletion.

Several studies are referenced in Section III.B.17 in which Urinary Tract Infections (UTIs), which are exceptionally unusual in adolescent boys and adult men, are positively associated with CKD in Nicaragua. We also raise questions regarding the implication of “UTI,” and subsequent the prescription of medications for flank pain and dysuria in adult male workers, and CKD. In our conversations with physicians and former workers, we have heard inconsistent information on the prevalence of UTIs, a variety of diagnostic practices, and the prescription of NSAIDs and aminoglycosides.

To clarify these inconsistencies we propose to conduct approximately 6-10 qualitative interviews with physicians at clinics in Chinandega and Leon likely to treat or diagnose UTI and/or CKD. The purpose of these interviews will be to identify beliefs and opinions regarding the diagnosis and treatment of UTI, and the prescription of medications. The

interview guide for each physician will include questions in the following areas: beliefs regarding UTI prevalence, knowledge regarding nephrotoxic properties of medications, perceptions of patient risk and patient expectations for treatment, availability and cost of medications, and opinions regarding the prevalence and etiology of CKD in Nicaragua. We anticipate that after conducting 6-10 interviews we should have sufficient responses that help us gain a more comprehensive and consistent understanding of the practices related to the diagnosis of UTIs and the prescription of NSAIDs or aminoglycosides. The exact number of people interviewed will be determined when we reach “saturation,” the point at which interviewees no longer provide new information, and our research questions are addressed.

Each interview will last 30-60 minutes. Questions will be primarily open-ended followed by a series of probes. Probes are a useful method for initiating questions in a non-threatening manner, creating a more conversational tone and helping to avoid polarizing or intimidating the interviewee. Probes are verbal cues and provide guidance to the person being interviewed. Where the meaning of a statement is ambiguous, the respondent will be asked to clarify a point; she may choose to expound on the point, present another story, or discuss feelings and impressions in greater detail. Points that are not discussed spontaneously are raised as part of the conversation. To the extent possible, we will probe responses by asking respondents to give an example. Examples force the respondent to be more specific and allow for a richer interpretation of text.

All interviews will be digitally recorded and permission to record will be requested at the start of each interview. Respondents will be assured that no names will be used and that while quotes may be used to illustrate themes in our findings, none will be ascribed to any individual. Interviewees will also be told that the interviewer will turn off the recorder if at any point the person does not wish a statement to be recorded.

Interviews will be initially analyzed using standard social science methods for analyzing mixed qualitative and quantitative data (Patton 2002). The interviews will be analyzed for themes (sometimes described as frequently repeated ideas) related to participant knowledge, experience, beliefs, and theories concerning these exposures and CKD. At this stage of the study, analysis of the interviews will be for the purpose of directing further research or intervention on hypotheses regarding CKD and association with UTI and/or medications, or abandoning these hypotheses for lack of evidence supporting them.

**Limitations and Uncertainties:** The limitation of interview data is that we rely on individuals as a source of information. However, this is also a strength of qualitative research in that the information provided by individuals is often not otherwise available. It is important to have well trained interviewers so that the information collected during interview sessions is as reliable as possible. To ensure this, our physician interviews will be piloted with physicians in Nicaragua.

## 9. Other Possible Activities

There are additional opportunities for study activities which our team has discussed or learned about in the process of developing our recommendations. Although we have not integrated them into our study plan as specific activities, we provide a list with a brief description so that readers of this report will have a more complete basis to provide input and suggestions:

Prospective cohort study among workers at ISA: our proposed study at is a retrospective cohort study, thus we are limited to information that has already been collected. However, beginning a prospective cohort study among current and new employees would enable us to collect additional information and would also help determine to what extent the problem of CKD among workers at ISA is decreasing or increasing. This latter question is of great public health importance in terms of evaluating some of the changes in work policies the company has initiated and for developing future interventions.

Collaboration with a second sugar company: Monte Rosa Sugar is located in the municipality of El Viejo in the department of Chinandega. Based on separate discussions with two company representatives, it appears that persons who work at Monte Rosa also have an elevated rate of CKD. The representatives further indicated their support for this initiative to bring resources to studying the problem and expressed an interest in participating in some way. Conducting certain parallel activities as recommended in this report at a second company in the same region would widen the scope from a single company and help strengthen interpretation of the results.

Assessment of cumulative exposure to lead: A limitation of biological testing for lead levels using blood is that it only provides information on recent exposure. Cumulative lead exposure can be assessed in bone using x-ray fluorescence. The procedure is impractical to carry out on a large scale, but we may want to test a smaller group if there appears to be any evidence of significant lead exposure based on either environmental or biological testing.

Collaboration with a new prevalence and case-control study of CKD: The group at UNAN-Leon CIDS, which conducts the Demographic and Health Survey in Nicaragua, in collaboration with the University of North Carolina has begun to conduct a seroprevalence survey with measurement of creatinine among approximately 3,000 residents in the municipality of Leon. They will then use that population as the sampling frame for a case-control study of CKD, which will collect both biological samples and questionnaire-based information. Results from these studies, which appear to have been rigorously designed, can also provide data from a different population. In addition to sharing results, providing funds to collect additional information beyond that currently planned (e.g., environmental sampling) could also increase the value of the study activities we undertake.

Initiation of prevalence studies in northeastern Nicaragua and Rivas: Tufts University School of Medicine, one of our sister schools in Boston, has an elective in which students provide medical care in Siuna, a town in northeastern Nicaragua where the primary economic activity used to be gold mining. Although this is the same activity as carried out in Larreynaga, the municipality in northwestern Nicaragua that along with Chichigalpa has the highest recorded rate of CKD mortality in the country, to our knowledge there is not believed to be a high rate of CKD in Siuna. It is possible that for very little additional cost, a cross-sectional prevalence study could be conducted there which would provide the first comparative data from outside the departments of Leon and Chinandega.

In addition, a physician affiliated with Boston University School of Medicine has close ties with the medical director of the regional hospital for Rivas, and they are both interested in conducting a prevalence study in that area. A modest amount of support could provide data from another area in the Zona del Occidente.

Most of these activities would require additional funding—some substantially more—and so may not be feasible. However, we hope that their inclusion here may spark additional ideas and may even lead to ideas for alternative sources of additional funding.

## V. CONCLUSION

The Dialogue process has created a unique opportunity to make great progress in the effort to determine the causes of the epidemic of CKD in Nicaragua and create the conditions for interventions aimed at preventing future cases. While many of the activities recommended in this report have been suggested by Nicaraguan investigators previously, they could not be implemented. In addition to the resources made available through the Dialogue process to allow the creation of an interdisciplinary team that possesses both a depth and breadth of expertise, the Dialogue has led to an unprecedented degree of access and cooperation among all parties. Our team has been promised unfettered access to the grounds at the ISA to conduct sampling, company employment and medical records, and cooperation of ASOCHIVIDA members in identifying sites of concern for sampling and providing work histories. In addition, both parties have been more than understanding of the reality that this work, while on a schedule faster than typical, will take time despite the fact that these are literally issues of life and death.

We appreciate the trust and cooperation shown to our team by all parties. It has allowed us to analyze the situation and propose an integrated set of activities addressing a range of hypotheses that we believe can move us a long way to the goal of stopping this epidemic. We know that this report is not perfect; it represents our best understanding at a particular point in time. However, we are still at an early stage in the process; we are confident that we will continue to learn, and we will modify our approach as we understand more. We have already benefited from the input of reviewers, and look forward to the same from the Dialogue partners and other involved and interested parties, to not only strengthen this report but all our activities on an ongoing basis. We have proposed an ambitious plan with important consequences for public health in Nicaragua. We hope and believe that, with the collaboration of the Dialogue partners and other involved and interested parties, we are up to the task.

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## Glossary

**albumin / albuminuria:** Human blood contains proteins that help the body function properly. *Albumin* is a particular type of protein carried by the blood. When the kidney is damaged, protein, including albumin, may leak from the blood into the urine. Measuring the amount of *albumin* in urine is a common sign of kidney disease. Too much albumin in the urine indicates potential kidney damage (see *proteinuria*.)

**analgesic:** Medication used as pain killer. Examples of *analgesic* medications include aspirin, acetaminophen (Tylenol), ibuprofen (Motrin, Advil), and naproxen (Aleve, Naprosyn). (see *non-steroidal anti-inflammatory drugs (NSAIDS)*)

**assay(s):** a procedure for testing the amount or composition of chemical or biological substances.

**associations (i.e., “epidemiologic association” or “modest association”):** Epidemiologists refer to the relationship between an environmental, occupational, or any other exposure and disease as an *association*. In studies that seek to identify a cause of disease, epidemiologists measure the strength of *association* between possible cause (often an environmental exposure) and disease using a variety of statistical methods. The results of such studies are sometimes described as having a strong, modest, or weak *association*. (Associations don’t always mean that one thing causes another. For example, carrying matches is associated with an increased risk of getting lung cancer, but matches do not cause cancer. Rather it is smoking that causes lung cancer.)

**bias / biases (recall bias):** Scientists attempt to do research that is objective and does not reflect any prior belief or desire about the results. *Bias* is a word used to describe methods of study that could lead to results that appear to favor a certain outcome, or prejudice against a certain outcome. Although not intentional, *bias* in study design threatens a study’s objectivity and is considered an error. *Recall bias* is a particular type of error epidemiologists anticipate and must control in study designs that require relying on people to report things that happened in the past, especially when some people being asked questions have the disease being studied (e.g., CKD) and others do not.

**biomarkers:** Biomarkers is another word for biological markers, or substances, found in human blood, urine or tissue. Some *biomarkers* can tell physicians and researchers about disease severity (e.g., blood creatinine levels), and other *biomarkers* can tell investigators about past exposures to chemicals or metals (e.g., bone lead levels).

**case-control study:** A type of epidemiologic study that typically identifies all the people with a particular disease in a population (“cases”) plus a sample of people from the same population (“controls”) and then looks at exposures (e.g., diet, occupation, behaviors,) in each group in order to find out whether the chance of developing the disease was greater for people who have had the exposure.

**cohort study (prospective cohort, retrospective cohort):** A type of epidemiological study in which people are grouped according to their exposures (e.g., occupational, residential,). Over time the cohort is followed for disease occurrence. When follow-up of the group, or *cohort*, begins nobody in the group has the disease being studied (e.g., CKD). Groups can be identified

and followed into the future (*prospective cohort*), or a group's past can be examined by looking at historic records (*retrospective cohort*).

**confounding factors (controlled, uncontrolled):** Risk factors for a disease are named risk factors because they are known to increase the risk of getting disease. Some risk factors for disease are associated with other exposures that also cause disease. They make it more difficult to tease apart the effects of a particular risk factor being studied on disease, and so are called *confounding factors*. Investigators must have some knowledge of confounders when designing studies so that they are adequately controlled. If studies do not *control confounding factors*, the study may be considered biased (see *bias*.)

**creatinine / serum creatinine:** A breakdown product of muscle that is filtered from the blood by the kidney. *Creatinine* can be measured in the urine, as well as in a component of blood called blood *serum*. Elevated creatinine levels can be used to help identify people with kidney disease.

**creatine kinase:** A particular kind of protein that is found in muscles. High levels of *creatine kinase* in the blood may indicate muscle damage.

**cross-sectional study:** A type of epidemiological study that examines the relationship between exposures and diseases in a defined population at one particular point in time.

**epidemiology:** the study of causes of disease in a population, generally through studies that collect information on disease and possible causes and then assess whether the amount of disease is higher in people who are exposed to these causes

**etiology / etiologies / etiologic agent:** The cause(s) of a disease, or, an exposure factor(s) that is strongly associated with disease.

**glomerular disease (glomeruli, glomerulus):** The *glomerulus* is the smallest structural unit of the kidney. It is made up of small blood vessels through which the blood is filtered to make urine. There are about 1 million glomeruli in each kidney. The earliest sign of diseases that affect the kidney is the appearance of protein in the urine; in the case of kidney disease involving the glomeruli, this protein in the urine is mostly comprised of albumin. (see *albumin*)

**glomerular filtration rate (GFR):** When the glomeruli are damaged or not functioning efficiently, the rate at which they filter blood is reduced. *GFR* is a measure of kidney function and low *GFR* is consistent with kidney disease. While it is possible to directly measure *GFR* using complicated and time-consuming tests, *GFR* is usually estimated using measures of serum creatinine (see *creatinine*) in addition to information about an individual's age, race, gender and other factors.

**glomerulonephritis:** Inflammation of the tissue in the kidney that serves as a filter, separating wastes from the blood. People with glomerulonephritis usually have protein and/or blood in the urine. (see *glomerular disease*)

**hematuria:** *Hematuria* occurs when red blood cells leak into the urine. Blood in the urine is a may be a sign of urinary tract infection but is also common in other kinds of kidney disease, including diseases affecting the glomerulus, also known as glomerulonephritis. *Hematuria* is not always visible to the naked eye, but may be detected by studying a sample of urine under a microscope or by other biochemical tests of the urine.

**incidence:** A measure of the frequency of new cases of disease that develop in a group over a specified time period.

**latency period:** the interval of time between the onset of disease that may not even be known to an individual, and the time when it is possible to clinically diagnose the disease.

**misclassification (exposure misclassification):** A type of error in epidemiologic studies where, for example, people who are healthy are defined as having disease, or, where people are incorrectly categorized as exposed or unexposed to an environmental hazard or other exposure.

**myoglobin / myoglobinuria:** *Myoglobin* is a protein that carries oxygen in muscle tissue and is very important for muscle function. *Myoglobinuria* occurs when the kidney is exposed to high amounts of myoglobin in the blood, as myoglobin is removed from the body by the kidney. High amounts of *myoglobin* protein measured in urine usually indicate muscle damage.

**nephrotoxic / nephrotoxin:** Toxic, poisonous or otherwise harmful to the kidney.

**non-steroidal anti-inflammatory drugs (NSAIDs):** A class of medications that have analgesic properties (i.e., they relieve pain) but also reduce inflammation caused by conditions such as arthritis. Examples of *NSAIDs* are ibuprofen, naproxen or Aleve.

**odds ratios / OR:** A measure used to compare the risk of disease in two groups, usually exposed vs. unexposed or high exposure vs. low exposure. An *odds ratio* of “1” implies that disease is equally likely in both exposed and unexposed groups. An odds ratio greater than “1” implies that disease is more likely in the exposed group. An odds ratio less than “1” implies that disease is less likely in the exposed group.

**prevalence / prevalence rates:** measures existing (as opposed to new) cases of disease in a group, usually at a specific point in time. (compare to *incidence*)

**proteinuria:** a condition in which urine contains an abnormally high amount of protein. Proteins in the blood perform a number of important functions, including helping protect the body from infection and helping the blood clot. As blood passes through healthy kidneys, they filter out the waste products and leave in the things the body needs, like proteins. Most proteins are too big to pass through the kidneys’ filters into the urine. However, when certain parts of the kidney are damaged, proteins from the blood can leak into the urine, causing *proteinuria*.

**random / randomly / random sampling:** Having no specific pattern, arrangement or predictable outcome. Random sampling is a method of choosing study participants to avoid *bias*.

**RfDs (reference doses):** An estimate of a daily exposure, or dose, of any given chemical to the human population (including sensitive subgroups) that is likely to be without risk of harmful effects during a lifetime. Generally used by regulatory agencies and in research.

**seroprevalence:** The overall frequency, or prevalence, of a biomarker within a defined group at one time, as measured by blood tests (serum). (See *biomarkers, creatinine*)

**toxicology:** The study of the harmful effects of chemicals on living organisms, including, understanding what happens once chemicals enter the human body, their transformation, duration of existence, interaction with human cells and tissue, and excretion via sweat, saliva, urine, etc.

**tubulointerstitial kidney disease:** Disease affecting the tubules of the kidney and/or the tissues surrounding the kidney tubules. Each glomerulus in the kidney has an attached tubule that drains the glomerulus of what will become urine. The tubule is the structural unit of the kidney where the composition of the urine is modified to increase the elimination of toxins that may be harmful to the body and to retain substances that are beneficial. Individuals with tubulointerstitial kidney disease typically do not have high amounts of proteinuria (Compare to *glomerular disease*)

**volume depletion:** The volume and composition of body fluid, including blood, is maintained primarily by the kidneys. A lack of normal intake or an increase in normal output of water can upset this balance, resulting in volume depletion (commonly called dehydration). This water deficit can be accompanied by an imbalance in the electrolytes, including sodium, potassium, and chloride, which must be maintained within narrow limits for proper body functioning. Blood and urine can be tested for signs of decreased body fluid volume. Late signs of volume depletion include low blood pressure and lightheadedness with standing.

# Appendix A: Health Studies Tutorial

## Summary Points

1. Questions (not goals) are driving force of research.
2. Implicit in every research question is a comparison.
3. Epidemiology: the investigation of disease and the factors contributing to the disease in a population.
4. We refer to the relationship between an exposure and disease as an association.
5. An investigation that is large enough to correctly observe what is actually happening (i.e., an association) has what is called sufficient "study power" or "statistical power."
6. Two reasons to collect and examine human blood or tissue samples include the investigation of biomarkers of exposure and / or biomarkers of effect.
7. Things to consider when designing an epidemiological investigation to study associations between exposure and disease, or causes of disease:
  - a. Timing: the cause must precede the disease (e.g., the rooster that crows before dawn does not cause the sun to rise).
  - b. Evidence that the relationship between exposure and disease is biologically plausible.
  - c. The strength of association between exposure and outcome. (Very rarely do we find definitive proof of an association or cause.)
  - d. The duration and amount of exposure and disease.
  - e. Control the confounding factors (factore de confusión).
8. Often there is interaction, or synergy, among multiple causes.
9. Sometimes there may be a cause that is very strongly associated with the disease but does not explain the high rates of disease in a population.
10. The task of epidemiologists to identify cause(s) that are sufficient (i.e. one, two or more things that are necessary to cause disease) to explain the high rates of a relatively rare disease in a population.

## Tutorial:

### Public health and medicine

These two areas of expertise are often confused. If a person has a disease, they go see a doctor for treatment. The doctor cannot always tell you how you got the disease, but they do their best to treat it. The goal of public health is to prevent disease in individuals via protection of the population. Generally, a certain amount of disease is normal. But when a person has a highly infectious disease (like influenza), or if many people have a disease at the same time, then we have a public health problem. Usually, they say, when public health works, we are not so aware of it. However, when things go wrong or when something does not work we begin to understand the value of a strong public health system. Public health is about preventing the disease in the population, while medicine is about treating the person. They rely on each other, but are not the same.

## Public health research

Public health research is distinct from experimental research because we do not have the ability to manipulate our research subjects. In real life, we cannot control the environment as we would in a laboratory and then observe responses to exposures.

Generally in public health research, specifically epidemiology, we look for patterns of exposure and/or illness in the population. In particular, we look for patterns that are not normal or common. In order to determine what is normal or not common, we must establish what is normal or common. Thus, in the heart of every research question there is a comparison. For example, if we ask: “Why are we sick?” We are also asking, “Why are we sick and not healthy?” Or, in the question: “Why are people in our community so sick?” We may also be asking: “Why are people in our community so sick compared with a different community?” Epidemiologists need to study both to answer the question. We ask: What are the differences between these groups of healthy and sick people?

It is true that sometimes we conduct because we want the results of research to bring us closer to achieving a goal. However, research is driven primarily by questions. Research questions drive research methods. And the truth is, we never know what the outcome of research will be ahead of time. If you are hoping for a particularly type of answer to a question, you may be disappointed.

Epidemiologists investigate the distribution of the disease and the causes of the disease in the population (for example, a neighborhood, city, department, or a country.) Therefore, the major objectives of epidemiology are to determine the extent of disease in a population, identify the factors that contribute to the disease, and evaluate the effectiveness of prevention and intervention.

In general, there are three types of concerns that drive research questions:  
... a preoccupation with a disease (for example, many people are sick)  
... a preoccupation with an exposure (e.g., living near a landfill / dump)  
... a preoccupation with understanding the relationship between the two (e.g., evidence that a greater number of people living near a landfill are sick compared with people who do not live near the landfill.) The last type of concern is the most common.

A well known example of exposure disease relationship is smoking cigarettes and lung cancer.

Smoking cigarettes causes lung cancer. We want to point out that this relationship is well documented in the scientific literature. There is ample evidence identifying an association. Further, we have a very specific exposure (smoking cigarettes) and a very specific outcome (lung cancer—not foot cancer or prostate cancer, but lung cancer.) There are many different types of cancer (colon, breast, lung, brain), with very different causes. When we are conducting a study we need to very clearly define both the exposure and the outcome.

We know that pesticides are a concern in this area. There are many different types of

pesticides known to cause a variety of very different health problems. For example, one type of pesticide is known to cause problems in the nervous system. From studies conducted on animals and in humans, we know it is possible that exposure to these types of pesticides may be associated with neurological problems. This relationship is biologically plausible.

Sometimes exposures are good. A diet low in iron leads to anemia. Sometimes disease is caused by not enough of something (e.g., nutrition, exercise).

To find answers to each type of question above, we rely on a number of different sources of information about exposure and disease. These include the literature that has been written by and reviewed by other scientists and published in professional journals, and we collect data.

### **Sources of data**

During a study sources of data collected may include:

- Death certificates and medical records: These sources often provide existing data on individual health status, cause of death, age of death, and diagnosis of disease. Epidemiologists look at such records for clues about the course of disease, possible causes, and other related conditions. When using these data sources we can often make comparisons within the population to identify patterns that help us understand the disease.
- Interviews and surveys: Asking people about their experiences, life, family, history, beliefs and opinions about the risks and treatments for disease help us identify factors that may contribute to disease and/or how disease is treated.
- Environmental sampling: Testing water, soil and / or the air samples for concentrations of potentially hazardous exposures. Sometimes we compare the locations of samples or analyse risk of exposure to humans based on our findings. Depending on the chemical characteristics, some remain in the environment for decades; others are broken down within hours. The same is true in humans.
- Human biomonitoring: We study human tissue samples (blood, urine, kidney biopsies, depending on the chemical compound of the study) for concentrations, as well as indicators of disease progression. Biomarkers of exposure v. biomarkers of effect (e.g., measuring creatinine in urine is a biomarker of disease, but it does not tell us anything about an exposure that may be associated with disease. These are different tests.)

Each of these sources of information tells us about exposure and / or illness, but not necessarily on the relationship between the two. For this analysis, we do usually statistical calculations requiring large number of participants, or “sample sizes”. Our ability to determine whether an association exists between exposure and disease, and whether this is a strong association, is based on getting large samples for analysis. If we do not have enough people, or samples (soil samples, water samples, blood samples), we may see an association that does not really exist, or worse, we may *not*

observe an association that really does exist. The ability of an investigation to correctly observe what is actually happening is called the power of the study, study power, or statistical power. This ability is determined by how large the study is (i.e., that sufficient samples are collected, or a sufficient number of people participate).

## **Epidemiologic study designs**

There are three “text-book” types of epidemiologic study design.

- **Case-control study:** Attempt to identify every person who is sick in an area, or population, and then compare them with people from the same population, looking for differences that explain why people are sick. Regarding the differences, I refer to diet, type of work, sexual activity, genetics... Then, if there is a difference in one of these areas among people who are sick, and everyone else, we measure the strength of the association. For this reason, for every case (or sick individual) at least one or more healthy individuals need to be included in, or participate in, the study. Such a study may include many of the data sources cited earlier. We always must be certain to have enough people so that the study has sufficient power, but on the other hand, we do not want to put many people in an unnecessarily uncomfortable position to participate, especially if it involves taking blood samples. Furthermore, the more people and samples involved, the more money and time such a study will cost.
- **Cohort studies:** A cohort is a group of people. A cohort study follows a group with certain shared characteristics (e.g., they work in the same place, they live in the same town, etc.), either into the future, or into the past by examining historical records. Like the case-control studies, cohort studies look for differences between those who become ill and those who remain healthy over time. The difference is that everyone at the beginning of the cohort study should be healthy. Then we observe who gets sick and how they differ from those who are not sick. What placed them at greater risk? This can be done by observing a group of people moving forward in time, or looking at the historical (e.g. medical history) of people in the past.
- **Cross-sectional study:** Finally, there is a type of study that examines an entire population at a single point in time, looking at both the diseases and exposures, regardless of when the disease began in relation to exposure. This type of design is the least likely to tell us reliable information about the relationship between exposure and disease for chronic disease, or diseases such as cancer that begin long before they are diagnosed. This is because you can't tell which came first, the disease or the exposure.

## **The Cause**

One secret of looking for “the cause” is thinking hard about things we assume. How do we really know that what we observe is true? There are several factors to consider when trying to identify a cause in epidemiological investigations.

- Timing. The cause must precede the disease. It could be a long time, ~ 20 years or more, or a short period of time such as minutes. Knowing when the disease began, and having a clear definition of the disease is very important. If we suspect that something in our drinking water is the cause of an illness that we began to observe 5 or 15 years ago, then the sampling of drinking water today not tell us what caused the disease. If we do not find anything in the water, it does not mean that what caused the illness was not something in the water. However, if we find something in the water, we may consider it likely that what is in the water now was also in the water before the disease. However, unless we have water samples before the illness began, we cannot do anything better than sample today and make inferences about the past. We also need a basis for examining certain water contaminants and their relationship with disease. The rooster crowing before dawn does not cause the sun to rise. So, we investigate whether the suspected cause precedes the disease.

Also the time of exposure to an individual can lead to a different outcome (i.e., exposure in a young child will have a different outcome than a full term in an adult). Timing of exposure during a lifetime is also important to consider.

- The strength of association between exposure and outcome. Very rarely do we find proof of causality. This is the nature of science. Instead we accumulate evidence. Over time, we learn more and more, and evidence sharpens our understanding. Specifically, the evidence accumulated is evidence of association. We examine the strength of the association, using statistical methods. Factors that contribute to the strength of association, using smoking and lung cancer as an example, include:
  - The amount and duration of exposure. Are people who smoke more cigarettes (2 packs / day) more likely to have lung cancer than those who smoke less (2 cigarettes / day)? Is there a relationship between length of exposure duration, intensity or the amount of exposure, and disease?
  - Not everyone who smokes gets lung cancer. But statistics have shown that people who smoke are more likely to have lung cancer. There is a strong association between cigarette smoking and lung cancer. If people did not smoke, we would see much less lung cancer in the world but we would still see lung cancer due to other causes weaker associations, but also contributors to lung cancer.
- Confounding. Epidemiologists spend a lot of time controlling confounding. The question we are addressing is: Is there anything that is related to exposure and the disease that could be interfering with our observation? For example: We know that smoking causes lung cancer. However, we also know that eating large quantities of fresh fruit and vegetables reduces the risk of cancer. (This is a negative association, in fact, this exposure protects against cancer.) If we study the association between smoking and lung cancer, and not look at the diet in our study population, we could find that everyone in our study who smoked also ate

lots of fruits and vegetables. This might make our results appear as if there is less of an association between smoking and lung cancer because people who smoked were not more likely to get lung cancer. To avoid this result, we have to ask people about what they eat, and then make sure that we compare people who smoked and ate fruits and vegetables to people who did not smoke and ate fresh fruit and vegetables—so the comparisons are the same, especially concerning confounders. Then we would see that there is actually a positive association between smoking and lung cancer. To avoid confounding, we need to know a lot about the disease, the exposures that may contribute to the disease, and have enough people to analyze the samples or groups with sufficient statistical power. This is an effort to "control the confounding factors."

### **Multiple causes**

As in the last example, more than one thing (diet and smoking) affects the disease. In fact, many factors may contribute to the outcome. This is particularly true of social factors (poverty) and environmental factors (multiple chemicals in the environment or the food we eat) that interact with themselves creating an effect that is greater than each of its parts. Multiple causes sometimes interact to have an added effect, sometimes called synergy or interaction. [Example in workshop was the ability of people to drive after one alcoholic beverage, and the ability to drive after one ibuprofen, but the inability of some people to drive after having both of these due to their combined effect.]

One of the causes people talk a lot because of our relatively recent ability to study them are human genes, or genetics. We are able to ask, Are some people more vulnerable to exposure (e.g., chemicals) because of their genes? Or is there something about people's genes that makes them have diseases that most people normally do not have (often people are born with such genetic diseases)?

### **Population attributable fraction**

Sometimes there may be a cause, or a factor known to be very strongly associated with the disease, but it does not explain the high rates of disease observed in a population. For example, you may know that there is a gene that puts some women at high risk of breast cancer. This gene is a cause of breast cancer. But there are many more cases of breast cancer than women in the world who have this gene. In the general population, for example in the United States, this gene is responsible for a small percentage of cases of breast cancer. Therefore, research into other causes of breast cancer continues, as this is a disease that affects many women and we have yet to identify a cause that explains the high number of diseases throughout the population.

That's why we say that there are individual risk factors such as genetic factors or certain diseases or medical conditions, and other environmental risk factors or occupational, such as industrial accidents. This is a problem in the case of renal disease, because we know that there are some individual risk factors that cause kidney disease but we do not

know if these causes of kidney disease are responsible for high rates of kidney disease that we see in the people of Nicaragua.

This example of breast cancer is also a good example of why we look at entire populations, not only when individuals carry out epidemiological investigations. The breast cancer gene is a rare exposure that is strongly associated with the disease. However, there are also much more common exposures whose association with disease are weaker, but are very common and therefore may contribute to many diseases in a population (e.g., skin cancer due to exposure to the sun).

The task of epidemiologists to identify the sufficient causes that are two or more things that are necessary for disease to occur that explain the rates of a disease in a population.

## Appendix B: Prior Investigations of Contaminants in Water Samples

Location of sample	Organochlorine Pesticides (ng/L)	Organophosphate Pesticides (ng/L)	Arsenic (µg/L)	Cadmium (µg/L)	Lead (µg/L)	Microbiologic (CFU/100mL)
<b>WHO Guideline</b>	Lindane: 2 µg/L (2000 ng/L), Dieldrin: 0.03 µg/L (30 ng/L)	N/A	10 µg/L	3 µg/L	10 µg/L	N/A
<b>PP – El Chorizo</b>	ND	ND	2.69	<LOD	<LOD	ND
<b>PE – El Chorizo</b>	Lindane (0.29), Dieldrin (<LOD)	ND	1.53	<LOD	3.12	Total coliforms (7. Fecal coliforms (7. Streptococcus (4.8 E. coli (5.3)
<b>PP – Adela</b>	Lindane (0.50)	ND	2.54	<LOD	2.61	ND
<b>PE – Adela Nueva</b>	Lindane (0.40)	ND	1.85	<LOD	6.3	ND
<b>PP – Espinoza</b>	ND	ND	2.71	<LOD	<LOD	ND
<b>PP – La Leona</b>	Lindane (0.43)	ND	2.23	<LOD	<LOD	ND
<b>PP – La Resistencia</b>	ND	ND	2.53	<LOD	<LOD	Total coliforms (2.
<b>PE – Paso Hondo</b>	Lindane (0.38)	ND	1.68	<LOD	16.83	Total coliforms (2.12) Fecal coliforms (3. Streptococcus (4.0 E. coli (2.11)
<b>PP – Las Americas</b>	Dieldrin (1.18)	ND	2.74	<LOD	<LOD	ND
<b>PP – La Puerta</b>	ND	ND	3.1	<LOD	16.47	Total coliforms (8. Fecal coliforms (7. Streptococcus (3.0 E. coli (2.2)
<b>PP – Tanque Rojo</b>	Lindane (0.29)	ND	2.76	<LOD	6.69	ND
<b>PP – La Casa Hacienda</b>	Lindane (0.32)	ND	2.99	0.23	7.02	ND
<b>PP – La Bloquera</b>	Dieldrin (1.59)	ND	2.43	<LOD	8.82	Total coliforms (1. Streptococcus (5.0

## Appendix C: Annotated References

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**(4) Cerdas M. Chronic Kidney Disease in Costa Rica. International Journal of Nephrology.**

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**(5) Garcia-Trabanino. [Nefropatía terminal en pacientes de un hospital de referencia en El Salvador] / “End stage renal disease in patients in a referral hospital in El Salvador.” Rev Panam Salud Pública. 12(3), 2002.**

Objective: To determine the conditions associated with end-stage renal disease in El Salvador and assess the epidemiology of the disease, especially in those without known predisposing factors.

Methods: This study is a case series from 1999-2000 of 209 patients in end-stage renal disease at a referral hospital who were worked up for chronic kidney disease.

Each new patient presenting with renal failure over 5 months was administered a survey and underlying causes for renal disease were investigated and recorded.

Findings: The following underlying conditions were identified:

- Diabetes, Hypertension, NSAIDS (in 30 patients, 15, 9)
- Renal Stones, Multiple myeloma (3, 1)
- Polycystic Kidney Disease (4)
- Nephrotic/Nephritic Syndromes (1, 1)
- Chronic Renal Infections (1)
- Chronic Obstructive Uropathy (1)
- Hemorrhage (1)

The remaining cases (67%) had no clear precipitant after work-up. Most cases were male and from rural, low-altitude areas.

This study helps rule out proposed theories, at least in this population of Salvadorians. Polycystic kidney disease, recurrent urinary tract infections, kidney stones, nephritic and nephritic syndromes were all investigated but found in only a small fraction of the patients. The authors also suggest: that the typical low-protein Central American diet may delay symptomatology of CKD.

**(6) Garcia-Trabanino. [*Proteinuria e insuficiencia renal cronica en la costa de El Salvador: deteccion con metodos de bajo costo y factores asociados*] / “Proteinuria and chronic renal insufficiency on the coast of El Salvador: detection with low-cost methods and associated factors.” *Nefrologia*. 15(1), 2005.**

The abstract is available in English.

Objective: To determine the prevalence of CKD in a small coastal community in El Salvador after screening by proteinuria on urine dipstick. The study was carried out with 291 male volunteers from a town of 832 men in a coastal region of El Salvador, only one previously diagnosed with CKD. Sampling method was not described . Men were screened by urine dipstick; if they were positive for proteinuria their creatinine and hemoglobin were checked.

Findings:

- Patients were screened by urine dipstick; only those who tested positive for proteinuria -- 46% -- had their creatinine checked
- 80% of these patients with proteinuria had only trace proteinuria on dipstick
- 13% of total study population had proteinuria (trace or greater) and creatinine > 1.5
- Diabetes and/or HTN were present in only one third of CKD patients.
- A control community at 500m elevation had 13% proteinuria and very low CKD

This community had high levels of CKD and proteinuria (trace or greater). No predisposing factors were found; the population was homogenous for agricultural labor/alcohol consumption/pesticide exposure. Major weaknesses of the study are possible enrollment bias and study design requiring proteinuria before testing for CKD.

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**(9) Murray D. Surveillance of pesticide related illness in the developing world. Intl J Occupational Envir Health.**

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**(10) USRDS. Annual Data Report: Incidence and Prevalence of ESRD - Chapter II. 1999.**

This document has not been summarized.

**(11) Hydrologic and hydro-geologic study in the area of Chinandega – Nagarote.**

This is a detailed study of the availability (in quantity and quality) as well as behavior and variation of hydrologic resources within a larger national program to revitalize agricultural activities in Nicaragua.

**(12 A-C) INETER. [Proyecto Magfor - INETER Estudios Hidrogeologicos en la region del Pacifico de Nicaragua] / “Magfor Project - INETER Hydrogeologic studies in the Pacific region of Nicaragua.”**

This document has not been summarized.

**(13 & 14) Zelaya Rivas F. [Prevalencia de intoxicacion pasiva por metales pesados y su correlacion anatomopatologica en enfermos de insuficiencia renal cronica de la region de occidente de Nicaragua 00-01] / “Prevalence of passive intoxication by heavy metals and their anatomopathologic correlation in chronic renal insufficiency patients in the region of western Nicaragua 00-01”**

Objective: Determine the prevalence of high lead levels in workers at ISA with CKD. In workers at Ingeniero San Antonio (ISA), 8 of 10 workers with CKD in a previous sample had been shown to have hair samples positive for lead. This study aimed to test a larger sample (42 patients with CKD) for hair and blood lead levels. Biopsy these workers in an effort to understand underlying pathologic process.

Methods: Sampling was not described and the consent rate was not reported. The definition of CKD was not reported.

Findings: 21 of 42 patients with CKD were found to have elevated serum or hair lead, but levels and methods were not reported. 14 of 15 biopsies were consistent with fibrosis; the final biopsy was found to have chronic glomerulonephritis with granular and linear IgG and IgM deposits. Survey questions seeking exposure information did not indicate potential pathways.. The authors discontinued the biopsies after 15 biopsies because the small fibrotic kidneys provide little information and lead to high rates of complications. The high prevalence of elevated blood/hair lead levels in this study was striking, but the lack of information on the methodology makes it difficult to interpret. The biopsy arm of the study might serve as a warning that biopsies of patients with advanced disease may be not be very productive and are potentially risky.

**(15) Rojas F. [*Estudio investigativo sobre seguridad ocupacional; realizado en el ingenio San Antonio en areas laborales de campo (Febrero - Abril 2003)*] / “Investigative study on occupational safety; conducted in the work fields of San Antonio sugar mill (February - April 2003).”**

This document was not summarized.

**(16 & 17) [*Enfermedades producidas por el calor*] / “Diseases caused by heat.”**

This document was not summarized.

**(18) Zelaya Rivas F. [*Promocion de estilos de vida saludable en trabajadores del campo del ISA*] / “Promotion of healthy lifestyles for the ISA field workers.”**

This is a report from Dr. Zelaya Rivas, medical consultant at NSEL (Nicaragua Sugar Estates Limited) describing the efforts made to preserve workers’ health. Figure. 1 summarizes probable causes of renal problems in field workers.

ISA (Ingenio San Antonio) concerns about health led to the development of a strategic plan to help preserve the health of workers, especially those in the fields. The strategic plan includes yearly operative plans that contribute to the improvement or maintenance of an adequate health status controlling modifiable risk factors for the emergence of diseases, such as: stress by heat, dehydration, malnutrition, poor hygienic conditions, repetitive urinary tract infections, use of anti-inflammatory medicine – self medication, as well as non modifiable risk factors such as genetic predisposition, chronic disease and race. Objectives of the strategic plan:

- Promote healthy life practices in field workers (weight control, recreation and physical exercise
- Encourage field workers to drink more than 8 liters of water, 2 to 3 ‘bolis’ with electrolites, and two cookies each day.
- Promote the use of protective equipment (chaps, satchels for ‘pailas’ and sharpeners, etc.)
- Promote healthy lifestyles in the workers’ families

Components of the yearly operative plans:

- Prevention of health problems
- Health check before employment
- Continued medical checks
- Prevention of health problems in the workers’ families

**(19) Zelaya Rivas F. [*Descripcion de las Principales acciones realizadas por el Ingenio San Antonio al problem renal de Nicaragua*] / “Description of the principal actions taken by the San Antonio sugar mill regarding the kidney problem in Nicaragua.”**

This document consists of a four-page table describing the actions taken, corrective measures, and results, followed by document 18 (with the exception of the figures that appear in

doc. 18), there is a critique of an article published by journalist Manuel Israel Ruiz Arias, associating CRI with a disease known as Bagazosis (due to inhalation of “bagazo”, thin dust resulting from cutting sugar cane).

This document contains a history of the different types of cultivars (cotton, sugar cane) related to CRI in Nicaragua as well as of the steps taken by ISA in order to try to understand the causes of CRI in the field workers.

Evidence for work in ISA not causing CKD includes::

- Spanish and Central American researchers have found a prevalence of 20 to 25% of children and teenagers with early manifestations of renal malfunction of genetic origin
- There doesn't exist a direct association between the disease and type of work
- Studies done by MINSA, INSS, National Assembly, Dept. of Labor, Human rights office and ISA have not found a cause in the production process that could be causing CKDI in workers nor in the general population
- This region is one where the working force is concentrated, so the number of CKDI cases is higher.

Further reading: [http://www.adnmundo.com/contenidos/enfermedad\\_laboral\\_la\\_100506.html](http://www.adnmundo.com/contenidos/enfermedad_laboral_la_100506.html)

**(20) Zelaya Rivas F. [*Condiciones socioeconomicas de trabajadores de corte de Cana del ingenio san antonio*] / “Socioeconomic conditions of San Antonio sugar mill sugar cane cutters.”**

This is a study aiming to understand the reasons why at the end of the 2004-2005 cane-cutting season, a number of field workers did not return to work in 2005-2006.

Description of the socioeconomic characteristics of cane-cutting workers in the 2005-2006 season in ISA, Chichigalpa.

- Demographic characteristics (age, origin, religion, education, marital status, socioeconomic situation)
- Home characteristics
- Family
- Personal: alcohol ingestion, drugs, smoking, distractions, social groups)
- Working conditions
- Nutrition history
- Workers' opinions on care received at the ISA hospital
- Quantitative cases study and qualitative interviews
- N=500 workers during the 2005-2006 cutting season
- Interviews completed in 8 sessions (detailed table of interview contents)
- The majority of workers live in extreme poverty
- Homes consist of two living areas, with 5 people
- Alcohol

**(21) Solis Zepeda GA. [*Impacto de las medidas preventativas para evitar el deterioro de la funcion renal por el Sindrome de Golpe por Calor en trabajadores agricolas del Ingenio San Antonio del Occidente de Nicaragua, Ciclo Agricola 2005-06*] / “The impact of preventative measures to avoid deterioration of renal function due to heat stroke in the**

**field workers at the San Antonio sugar mill in Western Nicaragua, 05-06 growing season.”**

This is a powerpoint presentation of a thesis (document #69).

Evaluation of the impact of preventative measures to avoid deterioration of renal function due to heat stroke syndrome in field workers at the Sugar mill San Antonio in Western Nicaragua, 2005-2006 season.

- Evaluation of the workers' knowledge of available preventative measure to avoid dehydration
- Determine the amount of water, hydrating bolis, and energy cookies that workers should consume before, during, and after the work day in order to prevent the accelerated progression of renal failure deterioration.
- Describe changes in renal function occurring during the work day
- Clinical controlled study with random assignation (case-control)
- N=405 workers (from among a total workers population of 2000).
  - treatment group, N=218
  - control group, N=187
- standardized interview, collection of blood and urine samples

Findings:

- Preventive measures to prevent damage to renal function by heat stroke syndrome have an important impact.
- The majority of patients know that drinking water, hydrating bolis, and eating energy cookies prevent changes in renal function
- Half of the workers in the treatment group know the amount of water, bolis and energy cookies they should consume to prevent renal damage
- In both treatment and control groups there is a tendency in elevated creatinine and urea nitrogen in blood at the end of the work day.
- Untreated (control) patients show changes in renal function

**(22) Silva T. [*Estudio socio-economico del nucleo familiar y del operario del corte de cana, Zafra 2007 – 2008*] / “Study of the socio-economic condition of the cane cutter and his family, 07-08 harvest.”**

This is a study from the Nicaragua Sugar Estates Ltd. (NSEL) and the San Antonio sugar mill (Ingenio San Antonio) hospital. One of the authors, Dr. Felix Arturo Zelaya Rivas, is the medical consultant who authored other reviewed documents.

Objectives: To describe socioeconomic and cultural conditions of sugar cane cutters' families (living and work conditions, nutrition, cultural characteristics, families' opinions on the ISA hospital and other healthcare facilities), with the aim of bettering the lifestyles not only of the workers but also of their families in order to maintain a certain level of satisfaction to work at NSEL.

Methods:

- Cross-sectional descriptive study (questionnaire)

- Study was done in different sectors of the departments of Chinandega and León.
- N=656 sugar cane cutters (out of a total number of 1094 workers)
  - 569 were from Chichigalpa, Chinandega (municipality of Chichigalpa, Dept of Chinandega)
  - 87 were from the municipalities of Quezalguaque and León
- Study used quantitative data (collected within a statistically representative subgroup) and qualitative data (observation and in depth interviews) analyses.
- Analysis used: Epi-info 6.04d

#### Findings:

- 60% (N=656) of the workers were interviewed. Preference was given to those living in the municipalities of Chichigalpa and Posoltega within the department of Chinandega, and to those living in León and Quezalguaque in the department of León.
- Workers in the dept. of Chinandega are older and have worked longer in the cane cutting process than those from León.
- Both Chinandega and León workers live in extreme poverty
- There exists a high prevalence of illiteracy in the children of workers living in León compared to those in Chinandega.
- Households are owned, and usually consist of one room where cooking, living and sleeping takes place.
- León workers drink water from either own or communal wells and 54% do not chlorinate drinking water. Most Chichigalpa workers have access to potable drinking water.
- Sewage water is used for irrigation and in urban environments, it runs in the streets.
- Trash is buried
- The main source of nutritional energy consists of carbohydrates (rice and beans). Proteins and lipids are acquired through eggs and cheese. The diet is poor in vitamins and minerals
- 52.9% of workers drink alcohol and the preference is beer. Little use of drugs but those who do ((N=2) are from Chichigalpa and consume marijuana. NOTE: tobacco is considered a drug by workers and 6 Chichigalpa workers are smokers.
- 20% of workers seek healthcare at the ISA hospital or other health centers. All of León workers and 12% of Chinandega workers complain about the difficulty (distance and road conditions) to reach the hospital and centers.

**(23) Zelaya Rivas FA. [Programa de oficina de responsabilidad social en salud del campo.] / “The office of social responsibility's program for the health of the country.”**

This document was not summarized.

**(24) Pastora Coca IV. [Monografía: Prevalencia de hematuria y/o proteinuria y su relación con algunos factores de riesgo en la población pre-escolar de la ciudad de León Abril-Agosto, 1998] / “The prevalence of hematuria and / or proteinuria and its relationship with some risk factors in the pre-school population of the city of León, April-August 1998.”**

This document was not summarized.

**(25) Zelaya Rivas FA. [Causas de enfermedad renal cronica en la poblacion laboral del ingenio San Antonio, Chinandega 00-01] / “Causes of chronic renal disease in the San Antonio sugar mill workforce, Chinandega 2000-01.”**

This document was not summarized.

**(26) Catrillo Sandino ME. [Factores de riesgo asociados a insuficiencia renal cronica en pacientes ingresados al departamento de medicina interna en el periodo de Enero 1998 a Julio del 2001] / “Risk factors associated with chronic renal insufficiency in patients admitted to the internal medicine department in the period from January 1998 to July 2001.”**

Objective: Determine which risk factors were most associated with CKD in the hospital’s population.

Methods: For each patient with non-terminal CKD sex- and age-matched controls were selected from elsewhere in the hospital. All consenting patients with CKD (65) were sampled along controls matched for age and sex. CKD was defined as creatinine > 1.2mg/dL; controls had creatinine <1.3 and GFR > 80.

Findings: Factors which reached significance included hypertension (OR 6.25), family history of CKD (OR 4), agricultural occupation (OR 3.83), previously diagnosed urinary tract infection (3.22) and well use (OR 3). Alcohol consumption, diabetes and rural residence were also non-significantly associated.

An association with urinary tract infection was found in almost half of cases. More detail on sex and age distribution of this group is needed. Given that the UTI had to have been previously diagnosed by a physician, this effect could be well underestimated. Family history was present in 18/65 cases.

**(27) Lacayo Casco A. [Hipocalcemia en pacientes con IRC ingresados al departamento de medicina interna de enero - diciembre del 2000] / “Hypocalcemia in patients with chronic renal insufficiency hospitalized in the internal medicine department from January to December 2000.”**

Examination of rates of hypocalcemia in patients (in León) with different stages of CKD and sees if hypocalcemia aligns with any clear risk factors: increased CKD, associated with higher levels of hypocalcemia.

**(28) Alonso C. [Insuficiencia renal cronica: un problema de salud publica actual en Chinandega, 1995-2001] / “Chronic renal insufficiency: a real public health problem in Chinandega, 1995-2001.” FETP – CDC.**

This document has not been summarized.

**(29) [Resultados de trasplante renal en ninos en Costa Rica: 28 anos de experiencia] / “Renal transplant results in Costa Rican children: 28 years of experience.”**

This document has not been summarized.

**(30) Henríquez CA. [La Nefropediatría en El Salvador] / “Pediatric Nephrology in El Salvador.”**

16 of 68 cases (24%) reported had an unknown cause. Another 5 were listed as tubulointerstitial disease and 10 were glomerulonephritis. These patients were pre-dialysis.

**(31) Lou-Meda R. [Diagnóstico del Estado de la Nefrología en Guatemala] / “Diagnosis of the current state of Nephrology in Guatemala.”**

General information on nephrology and pediatric nephrology infrastructure in Guatemala.

**(32) [Enfermedad Renal Crónica en niños: Aspectos epidemiológicos y de rehabilitación en latinoamerica y datos comparativos] / “Chronic kidney disease in children: Epidemiologic aspects and rehabilitation in Latin America and comparative data.”**

These data are from the 2007 report of the Latin America Pediatric Renal Transplant Registry. There is an extensive description of pediatric renal transplants in Central and South America including some data on CKD and end-stage renal disease (ESRD). Leading causes of pediatric ESRD throughout Latin America are: glomerulonephritis (33%), uropathy (25%), hypoplasia/dysplasia (18%) and unknown (14%).

**(33) Marra G. [Base de datos: Instrumento de recolección epidemiológica] / “Database: Tools for epidemiologic collection.”**

This document has not been summarized.

**(34) Cesar Delgadillo Cardenaogal. [Conferencia Centroamericana de nefrología pediátrica] / “Central American conference on Pediatric nephrology.”**

This is a description of the pediatric nephrology infrastructure in Nicaragua.

**(35) Sandoval Díaz M. [Desarrollo de la nefrología pediátrica en Nicaragua] / “Development of pediatric nephrology in Nicaragua.”**

This document has not been summarized.

**(36) Edefonti A. [Nefrología Pediátrica en Centro America - Resultados de un cuestionario] / “Pediatric nephrology in Central America - questionnaire results.”**

This document has not been summarized.

**(37 A-C) Marín Ruiz J. [Epidemia de Enfermedad Renal Crónica en la Región Occidental de Nicaragua, 2006.] / “Epidemic of Chronic Renal Disease in the Western Region of Nicaragua, 2006.”**

There are three similar PowerPoint presentations. The author is Dr. Jesus Marin Ruiz (CIVATOX – FFCCMM).

Objectives: Not given

Methodology: Data source: vital statistics

## Findings:

### National

- National morbidity ranges from 1200 to 1700 per year from 1996 to 2006.
- Nicaragua's national rates are lower than that for Japan, USA, Chile, Puerto Rico, Germany, Canada, and Venezuela (in order of decreasing rate).
- Morbidity rates are highest in Leon, Chinandega, and Managua. (SILAIS data)
- National mortality rates range from 7/100,000 in 1996 to 10/100,000 for years 2002-2006.
- Mortality rates are highest in Chinandega (25/100,000) and Leon (20/100,000). (SILAIS data)
- Mortality rates are consistently higher for men than women (2-6x higher).
- Mortality is higher for "ingenios" compared to "sin ingenios" for both men and women.

### Managua

- 60% of those affected by CRD are men.
- 55% are >45 years of age; 30% are 21-45 years old; and 15% are 1-20.
- 50% of cases had no related disease, 30% had diabetes mellitus, 15% had arterial hypertension, and 5% had another disease.

### Chinandega

- 90% of cases are men (1998-2005)
- 35% are >45 years old; 60% are 21-45 years old; 5% are 1-20 years old
- 91% have no related diseases, 4% have arterial hypertension, 3% have diabetes mellitus, 1% have another disease
- Stage 1 – 30%, stage 2 – 15%, stage 3 – 14%, stage 4 – 16%, stage 5 – 24%

### ESRD in ISA workers

- 11% are 61-80 years old; 55% are 41-60 years old; 34% are 15-40 years old
- 93% are from Chichigalpa, 4% are from Chinandega, 1% from Quezalguaque, 1% from La Paz Centro, and 1% from Villa Nueva
- 89% work in the field, 11% work in the factory

### Factors Related to CRD (last 8 years, 72 investigations):

- OR = 10 → Iija
- OR = 3-6 → alcohol, lead, pesticides, working longer than 8 hrs, dehydration
- OR = 2 or less → analgesics, cabrito, pesticide application, sugar cane harvester, plaque control
- OR < 1 → administrator, glucosamines

### Conclusions

- Different epidemiologic profile for Chinandega than Managua. In Chinandega, predominance of men that are younger/middle-aged (21-45 years old), and the vast majority have no related diseases.

### **(38) Marín Ruiz J. [*Plaguicidas*] / "Pesticides."**

This is a short ppt presentation listing data on intoxication by pesticides in Nicaragua. The number of cases and deaths by acute pesticide intoxication for years spanning 1990 to 2005 is given.

- 36% of females vs. 64% males are affected by acute pesticide intoxication (N=18,524)
- People aged 15-49 are more affected than those less than 15 or older than 50.

- The following pesticides, appearing only by their initials, O. F., FUM and HER are predominant in causing acute pesticide intoxications.
- A higher number of work-related intoxications was found in 1995, -96, -97 and -98, compared to accidental or intentional intoxications.
- 

**(39) Corriols M. [*Línea basal de colinesterasa*] / “Baseline cholinesterase.”**

This is a ppt presentation describing a study realized in 2004-2005 at the national level (Nicaragua) in order to evaluate levels of cholinesterase in workers that are prone to pesticide (that inhibit cholinesterase) intoxication.

**(40) [*Presentacion plomo*] / “Lead presentation.”**

This is a short ppt presentation describing different characteristics of 82 workers in ISA, in April 2002. Thirty nine (39) of the studied workers are healthy while 43 are sick with CRI.

**(41 A&B) [*Atención de la Enfermedad Renal Crónica*] / “Attention to Chronic Renal Disease.”**

This is a compilation of several slides on CKD epidemiology in Nicaragua, particularly mortality per 100,000 population.

**(42) Torres, C. [*Enfermedad Renal Crónica*] / “Chronic Renal Disease.”**

This is a summary PowerPoint presentation. It contains an excellent table using Ministry of Health data.

**(43) [*Enfermedad Renal Terminal en El Salvador*] / “End-stage renal disease in El Salvador.”**

This document has not been summarized.

**(44) Leiva R. [*Estudio Piloto de Prevalencia de Enfermedad Renal Crónica en El Salvador*] / “Pilot Study of Prevalence of Chronic Renal Disease in El Salvador.”**

This is a PowerPoint presentation entitled ‘Terminal Renal Disease in El Salvador.’ The presenter is Dr. Ricardo Leiva, Chief of the Nephrology Service of Rosales National Hospital in San Salvador, El Salvador. The presentation was given in Leon, Nicaragua on June 13, 2007.

**(45) PAHO. [*Propuesto de abordaje – Insuficiencia renal cronica en trabajadores agricolas del pacifico de Nicaragua*] / “Proposed approach - chronic renal insufficiency in agricultural workers from Nicaragua's Pacific coast.”**

This document has not been summarized.

**(46) [*2001 IRC en pacientes ingresados al servicio de Medicina Int*] / “Chronic renal insufficiency in patients admitted to the internal medicine department.”**

### Objective:

The purpose of this study is to identify risk factors that are implicated in the development of CRI in patients hospitalized in the internal medicine service of Escuela Oscar Danilo Rosales Arguello hospital (HEODRA) between January and December, 2000. Specifically, to test the hypothesis that those exposed to intensive labor, pesticides, NSAIDs and glucosamines have higher probability of developing CRI compared to those not exposed.

### Methods:

This is a case-control study. The hospital studied is located in Leon and serves patients in the surrounding urban and rural areas. Basic statistics, odds ratios, and 95% confidence intervals were calculated. Variables: age category, sex, urban vs. rural, case vs. control, occupation (intense labor and extreme environmental conditions – farm, factory, construction, etc. - vs. not exposed), glucosamine use (yes/no), NSAIDs use (yes/no), any pesticide use (yes/no). Cases: admitted patients in the nephrology division of the internal medicine service, azotemia (creatinine  $\geq$  1.2 mg/dl), anemia (hemoglobin - men:  $\leq$  14 mg/dl or women:  $\leq$  12 mg/dl), hyperuremia (uric acid levels  $>$  2.5-8 mg/dl), ultrasound information of CRI (renal size and thickness of cortex). Controls: admitted patients in another division of the internal medicine service during the same period, without CRI, 2:1 controls:cases. Data collection: standardized instrument, pretested, open- and closed-ended questions 165 cases, 334 controls

### Findings:

At GFR levels 20-35% of normal, some initial manifestations of CRI appear: infection or urinary obstruction (pg. 9).

Risk factors that produce a deterioration of renal function:

1) Low blood volume, a) secondary to diuretics, restricted ingestion of salt and water or digestive system loss such as vomiting, diarrhea, or physical activity at high temperature, b) secondary to decreased heart function, ascites with hepatomegaly, nephrotic syndrome, or others);

2) Pharmaceuticals, a) glucosamines, b) NSAIDs, c) contrast media;

3) Occupation – heat and physical effort lead to volume depletion, rhabdomyolysis and collapse of circulation;

4) Pesticides – organophosphates, carbamates, and herbicides

- There were more men among the cases (76%) than the controls (46%).
- Rural residence was more predominant among cases (58%) than controls (45%).
- Intensive labor at high temperature - OR = 5.44 (95% CI: 3.56-8.32)
- NSAIDs – OR = 4.16 (95% CI: 2.44-7.13)
- Glucosamines – OR = 3.74 (95% CI: 2.22-6.33)
- Pesticide exposure – OR = 9.28 (95% CI: 5.39-16.26)
- Stratified analysis:
  - Men, rural, occupational exposure – OR = 10.78 (95% CI: 2.81-48.58)

- Men, rural, NSAIDs – OR = 8.81 (95% CI: 2.29-39.80)
  - Men, rural, pesticides – OR = 10.13 (95% CI: 4.41-23.68)
  - Men and women, urban, glucosamines – OR ~ 8 (95% CI: 2.3-32.34)
- Increased odds due to occupation attributed to dehydration.
  - Increased odds with NSAIDs use in stratified analysis (men, rural) attributed to vasoconstrictory effects and resultant decreased renal blood flow.
  - Increased odds with pesticide exposure in stratified analysis (men, rural) explained by diminished diuresis, albuminuria, microscopic hematuria, and cylindruria.
  - Increased odds with glucosamines in stratified analysis (men and women, urban) explained by vasoconstriction and accumulation of pharmaceuticals in renal cortex.
  - Those persons exposed to heavy labor, pesticides, use of glucosamines and NSAIDs, have higher risk of developing CRI compared to those that are unexposed.

#### Recommendations:

- Develop a program for appropriate medication use to decrease nephrotoxic effects of pharmaceuticals.
- Correctly use protection to adequately prevent exposure to pesticides.
- Include CRI patients in primary care programs.

#### **(47) Marin Ruiz J. [Insuficiencia renal cronica: Cuadro clinico y situation epidemiologica en Nicaragua] / “Chronic renal insufficiency: Clinical diagnosis and the epidemiologic situation in Nicaragua.”**

Presenters: Dr. Jesus Marin Ruiz, Internist and Toxicologist & Dr. Jackeline Berroteran, MSc, Clinical Toxicologist

#### Objective:

To examine CRI in Nicaragua with respect to age, sex, geography, and specific causes: insecticide use, chronic diseases (diabetes), renal disease, and collagen disease. Specifically, this is a presentation with descriptive statistics about CRI with an emphasis on workers of ISA (ingenios azucareros?).

#### Findings:

- High mortality in Chinandega (Chichigalpa)
- Ingenios Azucareros workers
- Employees become ill within a few years of starting work
- Concerns about insurance coverage and treatment were raised

Source of data listed as “vital statistics, HISA statistics, CNPCST, database”.

- Slide 4: CRI cases in Nicaragua, 1996-2001, range: 1217-1721, steady '96-'98, increase '98-'00, decrease '00-'01
- Slide 5: CRI case rates in Nicaragua, 1996-2001, range: 25-40/100,000; decrease '96-'98, increase '98-'00, decrease '00-'01

- Slide 6: CRI case rates in Nicaragua by sex, 1996-2001, consistently higher for men than women
- Slide 7: CRI cases by region from SILAIS, 1996-2001, highest in Chinandega, Granada, Leon, Managua, and Rivas
- Slide 8: CRI case rates for men by skill level (?) from SILAIS, 1996-2001, consistently higher case rate for “ingenios” (skilled?) than “sin ingen” (unskilled?)
- Slide 9: CRI case rates for women by skill level from SILAIS, 1996-2001, consistently higher case rate for “ingenios” (skilled?) than “sin ingen” (unskilled?)
- Slide 10: Deaths due to CRI by sex in Nicaragua, 1996-2001, consistent by year for men, women, and overall; consistently higher for men than women
- Slide 11-12: Deaths due to CRI by product cultivated in Nicaragua, 1996-2001 (rice, corn, sugar, coffee), graphs are difficult to understand due to scaling of axes
- Slide 13: Type of contract for ISA workers with CRI ('98-'00) – vast majority are seasonal workers compared to permanent
- Slide 14: Age of ISA workers with CRI ('88-'00) – 41-60 years old: 50%, 15-40 years old: 30%, 61-81 years old: 10%
- Slide 15: Years of working with ISA for workers with CRI ('88-'00) – 21-60 yrs: 50%, 11-20 yrs: 30%, 1-10 yrs: 15%
- Slide 16: Residence of ISA workers with CRI, '98-'00 – 90% from Chichigalpa
- Slide 17: Area of work for ISA workers with CRI, '98-'00 – vast majority work in the field compared to the factory
- Slide 18: Factors of ISA workers with CRI, '88-'00
  - NSAIDs – 85%
  - Osteomuscular – 81%
  - Hyperuricemia – 80%
  - Alcohol – 75%
  - Glucosamine – 55%
- Slide 19: Clinical factors of ISA workers with CRI, '98-'00
  - Azoemia – 100%
  - Anemia – 97%
  - Hypertension – 96%
  - Hyperuricemia – 91%
  - Renal ultrasound findings – 87%
  - Slide 20: Heavy metals in consumed water, ISA, 2001 - lead: 50-55%, arsenic: 35-40%, cadmium: 30-35%

### Case-control analysis

- Slide 21: Bivariate comparison of those with CRI and healthy ISA workers, 2002 (cases – n=43; controls – n=39) – no substantial difference in residence or area of work. The cases are older, work more hours per day, and have worked more years than the controls.
- Slide 22: Work activities of ISA workers, 2002 – There is no difference between cases and controls with respect to work activities. For both groups, the predominant activity is sugar cane cutting, relative to irrigation, pesticides, and other.
- Slide 23: Hours of daily work for healthy vs. sick ISA workers, 2002 – The majority of sick workers work more than 8 hrs whereas the majority of healthy workers work fewer than 8 hrs (OR=13.33).
- Slide 24: Increased odds of disease for those that ingest alcohol among ISA workers (OR=7.55).

- Slide 25: 28% (12/43) of sick (CRI) ISA workers vs. 2.5% (1/39) of healthy ISA workers had a positive lead test (OR=18.88).
- Slide 26: Summary of risk factors for ISA workers, 2002 – lead – OR=18.88, >8 hr work day – OR=13.33, alcohol – OR=7.55, >15 yrs of work – OR=6, zafrero – OR=4, <6L liquids – OR=4.

**(48) Reyes Alvarez E. [*La IRC en Nicaragua*] / “Chronic Renal Insufficiency in Nicaragua.”**

This document provides an estimate of the magnitude of CRI in Nicaragua. (A complete translation of this document is available.)

**(49) Zelaya FA. [*IRC en Nicaragua: Descriptcion de una epidemia silenciosa 98-06*] / “CRI in Nicaragua: Description of a silent epidemic 98-02.”**

A complete translation of this document is available.

Objective: To describe the epidemiology of CRI in western Nicaragua between 1998 and 2006.

- To locate in time the CRI epidemic in Nicaragua
- To present the geographic characteristics of the origin of CRI cases
- To describe the personal characteristics of persons affected with CRI

**(50) [*Total de defunciones por ano en el registro civil de las personas de la alcaldia de chicigalpa, del 2000 al primer cuatrimestre 2007*] / “Total deaths per year in the civil registry of individuals in the Hall of chicigalpa, 2000 to first quarter 2007.”**

This is a table of the number of deaths per year for Chichigalpa for years 2000 through 2007, including the 1<sup>st</sup> quarter of 2007. The number of deaths for all causes is listed, as well as the total number of deaths due to CRI. CRI deaths are then stratified by sex. The percent of CRI deaths overall, female CRI deaths, and male CRI deaths out of deaths due to any cause is also provided by year.

**(52) [*Tendencia de la Enfermedad Renal Terminal (ERT) en el Mundo*] / “Global trend of end stage renal disease (ESRD).”**

This document has not been summarized.

**(53) [*Total de defunciones por año en el registro civil de la Alcaldia de Chichigalpa 00-07*] / “Total deaths per year in the civil registry in the Chichigalpa municipality.”**

This is a PowerPoint presentation about deaths in Chichigalpa. There are three slides.

**(54) Zelaya Rivas FA. [*IRC en Nicaragua: Discriptcion de una epidemia silenciosa 98-06*] / “CRI in Nicaragua: Description of a silent epidemic 98-02.”**

This document has not been summarized.

**(55-57) Torres C. 57: [*Estudio prevalencia ERC Chichigalpa, La Isla y Candelaria resultados preliminares*] / “Prevalence study of chronic renal disease in Chichigalpa, La**

Isla and Candelaria; preliminary results.”; 56: [*Estudio Prevalencia ERC Nor-occidente Nicaragua resultados preliminares*] / “Prevalence study of chronic renal disease in Northwestern Nicaragua, preliminary results.”; 57: [*Estudio Prevalencia ERC Nor-occidente Nicaragua resultados preliminares*] / “Prevalence study of chronic renal disease in Northwestern Nicaragua, preliminary results.”

These documents are PowerPoint presentations that were given by Dr. Cecilia Torres (CISTA) to the alcaldia, CAO, and to MINSA. Other presenters listed include Dr. Marvin Gonzalez (CISTA), Dr. Ramon Vanegas (CISTA), Aurora Aragon PhD (CISTA), Ingvar Lundberg PhD (U. Uppsala), and Catharina Wesseling PhD (UNA Costa Rica).

**(58) Torres Lacort C. [*Prevalencia de enfermedad renal cronica en el municipio de Chichigalpa, August 2008*] / “Prevalence of CRD in the municipality of Chichigalpa.”**

This is the report of Dr. Cecilia Torres’ CRD prevalence study in Chichigalpa, Nicaragua. The report was finalized August 2008. The other authors include Dr. Marvin Gonzalez, Dr. Ramon Vanegas, and Aurora Aragon PhD, associated with UNAN-Leon (Universidad Nacional Autonoma de Nicaragua), School of Medical Sciences, CISTA (Centro de Investigacion en Salud, Trabajo y Ambiente).

**(59) Torres Lacort C. [*Prevelencia de la ERC en la comunidad de "La Isla" y Reparto "Candelaria", Chichigalpa, Junio 2008*] / “Prevalence of CRD in "La Isla" and "Candelaria", Chichigalpa, June 2008.”**

This is the report of Dr. Cecilia Torres’ CRD prevalence study in Chichigalpa, Nicaragua, focusing on two neighborhoods – La Isla and Candelaria. The report was finalized in November 2008. The other authors include Dr. Marvin Gonzalez, Dr. Ramon Vanegas, and Aurora Aragon PhD, associated with UNAN-Leon (Universidad Nacional Autonoma de Nicaragua), School of Medical Sciences, CISTA (Centro de Investigacion en Salud, Trabajo y Ambiente).

**(60) Flor de Maria Amador Casador. [*Insuficiencia renal cronica en estado terminal (un estudio retrospectivo sobre su frecuencia diagnostico, tratamiento, evolucion y pronostico)*] / “Terminal chronic renal insufficiency (a retrospective study on its diagnostic frequency, treatment and prognosis).” Thesis.**

This report provides historical information about CKD in Nicaragua from more than 20 years ago. The author reports that records from the era before the Sandinista era (July 1979) have been lost, This study describes the end-stage renal disease (ESRD) patients in Managua in 1979, 1980, 1981 and 1982 at Bertha Calderon Hospital.

The majority of cases were from Managua (52/142), but there is a noticeably elevated number from Leon/Chinandega (35/142). No other province had more than 9 patients. This distribution is probably significant since Leon and Chinandega are distant from Managua. Male patients more often had CKD (64%) and were young: 57% were ages 20-50. 37% of patients lacked a clinical diagnosis (47% were diagnosed with glomerulonephritis; 17% were tubulointerstitial). Biopsies could not be performed due to lack of pathologists and facilities. Distribution of underlying causes and sex was not reported by province.

The data are sparse so caution should be taken in drawing conclusions, but there are already indications of the epidemiology we are now familiar with: a young, male-dominated disease from

the Pacific provinces. A potential confounder is selection/ascertainment bias due the high mortality rate for men killed in the revolution.

**(61) Icaza Rugama IL. [*Comportamiento clinico y epidemiologico en pacientes con insuficiencia renal cronica ingenio San Antonio, Chichigalpa Nicaragua*] / “Clinical and epidemiological behavior in patients with chronic renal insufficiency among San Antonio sugar mill workers, Chichigalpa, Nicaragua.” Thesis.**

This study is a series of 97 cases at Chinandega’s hospital. All but one patient was male, most were sugar cane workers and the most common age group was 48-59. This study confirms the profile of CKD cases: middle-aged, male agricultural workers.

**(62) Perez Lopez R. [*Modificaciones del balance hidroelectrolitico en trabajadores caneros del ingenio azucarero "San Antonio" sometidos a una solucion hidroelectrolitica*] / “Changes in fluid and electrolyte balance of the "San Antonio" sugar mill workers subjected to a hydroelectrolitic solution.” Thesis.**

This document has not been summarized.

**(63) Sanchez Acosta S. [*Modificaciones del balance hidroelectrolitico en trabajadores caneros del ingenio azucarero "San Antonio" sometidos a una solucion hidroelectrolitica INFORME FINAL*] / “Changes in fluid and electrolyte balance of the "San Antonio" sugar mill workers subjected to a solution hydroelectrolitic FINAL REPORT.” Thesis.**

Objective: To estimate the level of dehydration in sugar cane workers in San Antonio, Nicaragua. To assess the effectiveness of the implementing a electrolyte solution for the correction of electrolyte balance in the study population.

Methods: A quasi-experimental study was used. Field workers were chosen, as they spend the day working in high temperatures, with direct sunlight, and low intake of water, which predisposes them to acute renal failure. 15 workers were given salt solution (experimental group) and 15 others consumed water (control group). Both groups were evaluated in order to assess the effectiveness of this solution. Density measurements were made for urinary sodium, creatinine, urea nitrogen and uric acid.

Results: In conducting the experiment, the salt solution proved to be generally effective in correcting electrolyte disorders, such as those that occur with sodium (more common). The solution stabilized hydration levels in mild cases, but failed to correct high levels of dehydration due to the intense amount of labor the workers are subjected to.

Recommendations: Efforts should be put forth to educate people about the importance of maintaining adequate hydration and to drink large amounts of fluid (drinking average of 8 to 12 liters / day). Provide as much salt solution as possible as it is more effective than regular drinking water to avoid dehydration and electrolyte imbalance.

Due to high levels of sodium in the solution, workers demonstrated gastrointestinal side-effects such as diarrhea, nausea, thirst and hunger. A solution with lower levels of sodium is recommended.

It is important to conduct further research to expand the knowledge of the salt solution and its effect on the prevention of Acute Renal Failure.

**(64) Uriarte Barrera ED. [*Exposicion a cadmio e insuficiencia renal cronica en trabajadores del ingenio San Antonio*] / “Exposure to cadmium and chronic renal insufficiency in San Antonio sugar mill workers.” Thesis.**

Cadium is a heavy metal that, in its natural form, can be found in the earths crust and is released in volcanic emissions. It can cause nephrotoxicity. In Nicaragua, there have been little studies regarding CRI and none that explore the potential of cadmium as a possible risk factor of nephrotoxicity. The authors seek to provide knowledge on cadmium exposure in areas with a high prevalence of CRI.

Objective: To describe the current Cd exposure from water ingestion and tobacco smoke inhalation in farm workers that suffer from CRI in the region of Ingenio San Antonio, Nicaragua.

Methods: There were 30 study participants (workers in Ingenio San Antonio). 15 men were used as a reference group, and 15 were cases (diagnosed with CRI). All of the participants in the study have lived in Chichigalpa for the past 30 years. The majority are male. The main form of data collection was through a specially designed questionnaire. Chronic exposure to volcanic emissions was assessed from the years of living in the area.

Results: The concentration of proteinuria and plasma creatinine is significantly higher in the cases than in the controls. The mean exposure to Cd through tobacco smoke inhalation is higher in the cases than the controls (  $0.75 \pm 0.56$  ug/day vs.  $0.46 \pm 0.37$  ug / day mean  $\pm$  SD) , although the difference is not statically significant ( $p=0.21$ ). The cumulative exposure follows a similar pattern ( $p=0.37$ ). The exposure to Cd through ingestion of water is significantly higher in the cases that are smokers than in the reference group ( $p=0.02$ ). Overall, those cases that are smokers have a higher exposure to Cd than the reference group, although this is not significantly significant ( $p=0.09$ ). Nutritional status does not seem to be a predisposing factor in Cd absorption. The symptom of bone pain is higher in the cases.

Conclusion: The exposure to cadmium through water ingestion and tobacco smoke inhalation shows a tendency to be higher in those that suffer from CRI. However, there needs to be additional studies involving a larger number of participants in order to confirm or refute this association.

**(65) Morales Molina HL. [*Cambios en el calcio ionizado y del estado acido base en pacientes con insuficiencia renal cronica sometidos a transfusion hematica*] / “Changes in ionized calcium and acid base status in patients with chronic renal failure undergoing blood transfusion.” Thesis.**

This document has not been summarized.

**(66) Pilar Martinez Gurdian E. [*Comportamiento clinico y epidemiologico de la Glomerulonefritis aguda en ninos menores de 12 anos HEODRA Leon*] / “Clinical features and epidemiology of acute Glomerulonephritis in children under 12 in HEODRA Leon.” Thesis.**

This document has not been summarized.

**(67) C. Lopez Arteaga Y. [*Historia laboral agricola como factor de riesgo para deterioro de la funcion renal en el occidente del país*] / “Agricultural work history as a risk factor for deterioration of kidney function in the western part of the country.” Thesis.**

This document has not been summarized.

**(68) Espinoza CA. [*Relacion entre patron de ingesta de licor en hombres del area rural y deterioro de la funcion renal*] / “*Relationship between patterns of alcohol intake in rural men and deterioration of renal function.*” Thesis.**

This document has not been summarized.

**(69) Solis Zepeda GA. [*Impacto de las medidas preventivas para evitar el deterioro de la funcion renal por el síndrome de golpe por calor en trabajadores agricolas de ingenio San Antonio del Occidente de Nicaragua, Ciclo agricola 2005-2006*] / “Impact of preventive measures to avoid deterioration of kidney function due to heat stroke syndrome in agricultural workers of the San Antonio sugar mill in Western Nicaragua, agricultural season 2005-2006.” Thesis.**

This thesis corresponds to document #21, a powerpoint presentation of this document, with more detail on the study consisting of a case/control study aimed at determining whether heat stroke syndrome has an impact in renal function deterioration.

**(70) Narváez Caballero A & Morales Mairena E. [*Epidemiología de las defunciones por Enfermedad Renal Crónica en los últimos 20 años (1988-2007), en el Municipio de Chichigalpa-Chinandega*] / “Epidemiology of deaths from Chronic Renal Disease in the past 20 years (1988-2007), in the Municipality of Chichigalpa-Chinandega.” Thesis.**

This is a thesis by two students at UNAN-Leon, School of Medical Sciences.

Authors: Aaron Narvaez Caballero, Erinaldo Morales Mairena

Mentors: Dr. Cecilia Torres Lacourt, Family Medicine, Diploma in Occupational Health; Dr. Andres Herrera Rodriguez, PhD, Professor of the School of Medical Sciences  
UNAN-Leon, College of Medical Sciences, CISTA, Leon, November 2008

- ANAERC = Nicaraguan Association of those affected by CRI
- The background section mentions the pediatric situation in North America, Europe, Argentina, and Mexico.
- In Latin America, about 150,000 persons have been put under renal replacement therapy in the past few years.
- In 1995, 97 CRD patients were diagnosed in Ingenio San Antonio, the majority of which were sugar cane cutters and men. 34% were in stage 4.
- In another study of children in Managua (1983), the majority were male and 6-10 years old.
- High mortality, progressive disease which does not present symptomatically early in the evolution of the disease
- In 2005, in Nicaragua, 4-6 persons died per month of this disease.

### **Objectives:**

- General: To understand the epidemiologic profile of deaths due to CRD in the last 20 years (1988-2007) in the municipality of Chichigalpa-Chinandega
- To describe sociodemographic characteristics of those that died as a result of CRD
- To understand the CRD mortality rate in the municipality of Chichigalpa during the period 1988-2007
- To quantify the social impact of CRD by calculating years of productive life lost in the municipality of Chichigalpa.
- To calculate the sub-registry of the different sources of information
- Pathophysiology is discussed

### **Methodology:**

- Descriptive, cross-sectional study of 855 deaths due to CRD in the municipality of Chichigalpa, of which 88.5% are men and 11.5% are women.
- Study population: population of Chichigalpa between 1988 and 2007
- Inclusion criteria: deaths due to CRD in the civil registry and the MINSA registry, '88-'07
- Exclusion criteria: the deceased of other causes of death
- Data source: secondary, archives of the mayor's office of Chichigalpa and MINSA  
Only 36% overlap in reporting of deaths by MINSA and alcaldia (mayor's office of Chichigalpa)
- Collected information: age, sex, place of residence, civil state, occupation, education level, cause of death
- Year categorization: the 20 years were grouped into five categories of four years each – 1988-1991, 1992-1995, 1996-1999, 2000-2003, 2004-2007); there were no data available from MINSA for the first period, so only the mayor's office data were used for this period.
- Base population estimates: estimated population = initial population \* (1+rate of annual increase)<sup>number of years since the initial population census</sup>
- Years of productive life lost: estimated years of life at birth – age at death

### **Findings:**

- Majority of cases are men, 30-59 years old, and are agricultural workers (42%)
- Increasing overall mortality rates; increasing CRD-specific mortality rates (60/10,000 persons in 2003-2007); increasing proportion of all deaths are attributable to CRD (26.5% in 2003-2007)
- 14,790.7 productive years of life lost due to CRD over the past 20 years
  - Annual years lost has been constant for women over the 20 year period
  - Annual years lost has increased for men over the 20 year period
- 855 deaths in 20 year period
- Overall for 20 yr period, 88.5% male; proportion of males increased from 82% in 1988-1991 to 92% in 2003-2007
- Peak age overall for all 20 years and both sexes was 40-49 and 50-59 years old. Although for men, the majority of deaths (25%) occurred in the 40-49 yrs age range, whereas the majority of women dying from CRD (36%) do so within the 70-79 yrs age range. Men die much earlier than women from CRD.
- Epidemic of CRD with high mortality rates
- High social and economic cost for the community of Chichigalpa
- It is necessary to conduct studies to identify causal risk factors of this epidemic.

## **Recommendations:**

### **MINSA**

- Improve programs for early health care of CRD and amplify coverage and diversify offering of renal replacement therapy.
- Improve statistics system for better epidemiologic control of diseases.
- Promote mental health care for those with CRD and their families for the high emotional burden associated with this chronic disease.

### **Alcaldia**

- Eliminate the tax in order to stimulate reporting of deaths and improve the passive collection of information

### **UNAN-Leon**

- Designate funds to realize new studies that have as an objective to continue to explore the causes and related determinants of CRD as the beginning of improving life conditions of this population.

### **(71) Pollutech Intl. Limited. Analysis of water samples for metals and pesticides - Nicaragua Sugar Estates Limited.**

This document has not been reviewed.

### **(72-75) Evaluation of heavy metals - August 2001 CIRA; 2001 Evaluation of the bacteriological water in August 2001 by the CIRA; 2001 Evaluation of the coliforms in water in August 2001 by the CIRA; 2001 Evaluation of pesticides in water in August 2001 by the CIRA.**

This is a report from UNAN water samples analysis from 12 wells in ISA. Analyses include: organochlorides, organophosphates, cadmium, lead, arsenic, mercury and bacteriological content.

### **(76) University of Atlanta Center of Natural Water Resources. [*Analysis de manto acuifero en quimicos bioambiental tierra y agua*] / "Analysis of land and water chemicals in the mantel acuifier."**

This document has not been summarized.

### **(77) Lu C. Pesticide exposure and risk website.**

This document has not been summarized.

### **(78) [*Resumen de las actividades efectuadas por el MINSA ante la Problematica de los Enfermos con IRC*] / "Summary of activities conducted by MINSA dealing with the problems of patients with chronic renal insufficiency."**

A complete translation of this document is available.

### **(79) Garcia Trabanino R, et al. [*Nefropatia terminal en pacientes de un hospital de referencia en El Salvador*] / "End-stage renal disease in patients at a referral hospital in El Salvador." Rev Panam Salud Publica. 12(3), 2002.**

This document has not been summarized.

**(80) Sigma-Aldrich. Material Safety data sheet. 2006.**

This document has not been summarized.

**(81) Yearout R, et al. Impact of DBCP on participants in the agricultural industry in a third world nation (an industrial health, safety case study of a village at risk). Intl J of Industrial Ergonomics.**

This document has not been summarized.

**(82) Centro de Informacion Vigilancia y Asesoramiento Toxicologico (Dra. Jacqueline Berroteran). [Informes Sobre Intoxicaciones Agudas por Plaguicidas en Nicaragua] / "Reports About Acute Pesticide Poisonings in Nicaragua."**

This document has not been summarized.

**(83) Callejas Callejas L, Medrano CA, Mendoza Canales B. [Estudio de IRC en trabajadores no relacionados al cultivo de la caña de azúcar, Chinandega, Julio-Agosto 2003. Resultados preliminares] / "Study of the IRC in workers non-related to sugar cane, Chinandega, July-August 2003. Preliminary Results."**

**Objectives:**

Estimate prevalence of Chronic Renal Disease in non sugar cane workers in Chinandega, determine risk factors and develop prevention measures.

**Methodology:**

Cross-sectional population based study. The population was extracted (convenience sample) from the general male population in Corinto and El Realejo communities mainly. Initial N= 343. 16 were excluded because they had past history of working in the Ingenio, and one was because there was missing information. Final N (after inclusion criteria)=326.

An individual survey was administered looking for sociodemographic factors, environmental and occupational factors (pesticides use) and individual (alcohol, NSAIDs consumption and history of DM and HTN). Case was defined as Crs  $\geq$  1.5mg/dl

**Findings:**

There were 24 cases (7.5% of the sample), of whom, 21/291 lived in Corinto (sea port) and 3/23 lived in El Realejo. There is a slide on Current Occupation all the job titles refer to work done at the sea port (estibador, operario, portalonero, winchero, aguador, cheque, bodega, etc). The majority are estibadores 160, and there are 12 cases among them. Another slide describes

Occupational Factors where other professions are recorded (jornalero, comerciante, agricultor, ganadero). All occupations except comerciante, have Relative Risks around 2-4 and CI from 1.08 to almost 10. Another slide summarizes the types of crops grown; only beans has a RR of 3.6 (IC95% 1.40-9.46). Note: Caution should be used interpreting results due to small number of cases. Among the cases 3 had HTN, 2 DM, 9 IUR (Recurrent urinary Infection?), 2 CA. VU (urinary track cancer?), and 3 family history of IRC. 24/24 cases have a history of alcohol consumption (and 268/302 non cases). Analyses were performed by type of alcohol consumed (beer, lija and flor de cana). Only lija is statistically significant (20/24 among cases and 146/302 among non cases), RR= 4.82 (CI 95% 1.68-13.7).

Overall prevalence was 7.5%. Interesting classification scheme for occupation in two sectors (sea port and agriculture).

**(84) Cooper Mendoza DL, Chavarria Urbina GM, Rodriguez Espinoza MS. [Prevalencia de azoemia como indicador de Insuficiencia Renal en los pobladores del Municipio de Telica y Nagarote del Departamento de León en el periodo de Abril-Septiembre del 2008] / “Prevalence of azoemia as an indicator of Renal Insufficiency in the inhabitants of Telica and Nagarote Municipalities of Leon’s department in April-September 2008 period.” Thesis.**

**Objectives:**

Estimate prevalence of Chronic Renal Disease in Telica and Nagarote; determine the distribution of stages of disease; describe demographic and occupational characteristics; assess risk factors related to residence and occupation.

**Methodology:**

Cross-sectional population based study. Probabilistic sample using RSAMPLE of EpiInfo. There was no information about participation rate/refusal rate The sample size was based on an estimated prevalence 26%. **N=400** (200 in Telica, 100 urban/100 rural; 200 in Nagarote, 100 urban/100 rural). An individual survey was administered examining sociodemographic factors (age and gender), environmental and occupational factors (type of labor, type of crop, type of occupational activity) and individual characteristics (alcohol, drugs, smoking, water consumed during a day of work, NSAIDs (type and quantity), injectable antibiotics (type and frequency), pesticides (used it, type, intoxications), history of DM, HTN, repeated UTI, malaria, leptospirosis. Lab variables: creatinine and estimation of GFR with the Cockcroft-Gault formula, proteinuria (dipstick, yes/no), leucocyturia (yes/no), hamaturia (yes/no). Weight and height self reported. (Questionnaire and informed consent available in the document)

Definition of CKD clinical stages based on GFR, with stage 1 having normal GFR but kidney damage. (Note: contradictory description of stage 1 as the absence of disease.)

**Findings:**

305 people stage I, 67 (17%) Stage II, 20 (5.1%) stage III, (0.3%) Stage IV, (0.5%) Stage V. See graph 6 for distribution in Nagarote and Telica.

Participation of 15-55 years old by decades well distributed (older than 55 less represented). 56.8% of the sample are women. The most common occupation is "Other" (65.5%) which refers to health and education workers and housewives. Jornaleros (11.8%), Obrero (building worker?) (9.2%), agriculture (7.9%). See graphs 7,8,9,10, 11, 12, 13, for assessment of occupation; alcohol consumption; smoking; pesticide use; drugs use; personal history of malaria, diabetes, HTN, UTIs, leptospirosis; and family history WITH IRC. Overall, the prevalence of CKD was 5.9% (Stages III-V) in Nagarote and Telica. The data are available for further analyses.

**(85) Kim S-J, Gil H-W, Yang J-O, Lee E-Y, Hong S-Y. The clinical features of acute kidney injury in patients with acute paraquat intoxication. Nephrol Dial Transplant. 2002; 24:1226-1232.**

This document has not been summarized.

**(86) Senanayake N, Gurunathan G, Hart TB, Amerasinghe P, Babapulle M, Ellapola SB, Udupihille, Basanayake V. An epidemiological study of the health of Sri Lankan tea plantation workers associated with long term exposure to paraquat. British Journal of Industrial Medicine. 1993, 50:257-263.**

This document has not been summarized.

**(87) Jiron Romero NdJ, Reyes Alvarez E (Instituto Nicaraguense de Seguridad Social, Direccion de Garantia de Calidad). [Guía de practica clinica para prevencion, abordaje diagnostico temprano y tratamiento sustitutivo de la enfermedad renal cronica (ERC).] / "Clinical practice guide for prevention, early diagnosis, and replacement therapy for chronic renal disease." February 2008.**

This document has not been summarized.

## Appendix D: Peer Review Comments and Responses

**Dean Baker, MD, MPH  
Professor and Director  
Center for Occupational and Environmental Health  
University of California, Irvine, USA**

### **I. CKD in Nicaragua: State of Knowledge**

1. The report provides a good summary of previous efforts describing the CKD prevalence and mortality rate in Nicaragua. The report also presents a reasonable overview of the potential causes of CKD.

**Response:** We thank Dr. Baker for his positive comments.

2. Conclusions regarding CKD prevalence and mortality patterns are reasonable, e.g., higher in Chinandega and Leon, younger age, primarily men, association with manual and not service work. Assuming the quality of the original data is robust enough, these patterns should be used to focus future investigations.

**Response:** We agree wholeheartedly and will use existing data and any newly available data to focus our future studies.

3. More focus on building on existing studies and capacity. An important future study activity is to critically evaluate the recent and ongoing studies, develop strategies to build on the high quality studies, and facilitate cooperation among investigators.

**Response:** We have added more text to the report regarding our assessment of the quality of the completed studies. However, the motivation for Dr. Baker's comments appears to be the identification of potential Nicaraguan collaborators to facilitate cooperation among the groups of investigators currently working on CKD. Although the report listed many investigations, we are aware of only two groups --UNAN-CISTA and UNAN-CIDS in collaboration with the University of North Carolina -- who are currently conducting research in the area. We are also aware that we may be unaware of other active researchers and hope to be able to identify other potential collaborators through public presentations we will be making in December. Finally, we have are proposing a Scientific Advisory Board which will likely be composed of two US and two Nicaraguan investigators and that will provide another important vehicle for input.

4. Report needs to specify if information that was not available for existing studies is critical to obtain to support conclusions. This information could be important if there is disagreement about epidemiological patterns as summarized in the report.

**Response:** The section on limitations has been expanded to addresses the lack of details regarding the existing studies. As the section now describes, this limitation is particularly acute for the control of confounding variables.

5. The report does not discuss the availability and quality of the existing medical records. This may be part of the proposed pilot work and result in a report that critically analyzes

the available data. This report should be reviewed by CAO and participants before detailed work proceeds.

**Response:** We agree that a key task is to conduct a more comprehensive review of the quality and availability of existing medical and other records and have extended our pilot phase by one month to accommodate these activities. We also concur that a report of our pilot phase findings should be drafted and reviewed by CAO, dialogue participants, and our Scientific Advisory Board before work proceeds.

## II. Specific hypotheses

1. Volume depletion and muscle damage should not be listed as “susceptibility factors,” but rather as occupation factors.

**Response:** We did not mean to imply that volume depletion and muscle damage represent individual susceptibility factors. Rather, as Dr. Baker correctly infers, they may represent consequences of exertion in an extreme environment that may exacerbate underlying CKD or lead to synergistic effects with other factors. Although some have suggested that certain individuals have a genetic predisposition to muscle damage with extreme exercise, which might represent an individual susceptibility factor, such factors are not the focus of our investigation.

2. The list of agrichemicals that will be evaluated focuses on those used for sugar cane production. Pesticides such as DDT as well as chemicals used for cotton production should also be evaluated.

**Response:** We agree and will evaluate all relevant agrichemicals used for sugar and cotton production, as well as for other crops grown in the region. The Ministry of Agriculture is a good source of information on the historical use of these chemicals.

3. In addition to the active component of each agrichemical, inert ingredients, impurities and contaminants should also be evaluated.

**Response:** We agree and will attempt to identify both active and inactive ingredients, impurities, and contaminants in each agrichemical.

4. The evaluation of heavy metals is given too much emphasis in the sampling approach because this is an unlikely hypothesis.

**Response:** While heavy metals are a less likely hypothesis, their assessment is not very costly and will address at least some of the concerns of the parties, thereby increasing the acceptance of our project. In addition, because there is a fair chance that a multifactorial etiology may be at work, exposures that themselves might not be likely causes must be viewed differently when considered as a factor that causes CKD in combination with volume depletion, for example.

5. It is appropriate to discuss all hypotheses, but the dialogue participants should not get distracted by those that do not follow the epidemiological pattern observed (e.g., the hypotheses of medications, alcohol, kidney stones, structural kidney disease, glomerular disease associated with diabetes and hypertension, urinary tract infections, and genetics).

**Response:** At this stage, we think that it is very important to present all possible hypotheses as well as our assessment of their likelihood. As mentioned above, some of the motivation is to address the concerns of the parties in order to increase the acceptance of the results. We also think that a study that reviews medical records but does not directly address the issue of diabetes or hypertension, the major causes of CKD worldwide, will be criticized by the scientific community. Finally, as noted above, the possibility of multifactorial etiology makes some of these hypotheses more plausible, such as nephrotoxic medications combined with volume depletion.

### III. Recommended Activities

1. An important task is to conduct a critical analysis of prior studies and existing data and records.

**Response:** Without obtaining more detailed information, we do not believe that we can conduct a more critical analysis of prior studies than has already been done. However, we are planning to conduct a detailed analysis of existing employment, occupational and medical records during our pilot study.

2. Approach should encourage coordination and facilitation of all research pertaining to CKD, and not just research to be conducted by the BU team.

**Response:** We agree and are willing to participate in and encourage activities that facilitate coordination of all research on CKD. Meetings that we have set up for December will hopefully engage academics, physicians, government agencies, and other employers.

3. Environmental Sampling:

- a. Environmental sampling can provide a “screening level” understanding of whether immediate action needs to be taken, but cannot provide definitive information regarding the cause of CKD because it is essentially like looking for a needle in a haystack.

**Response:** We agree and will make dialogue participants aware of the possibility of false negatives.

- b. Rationale for the sample size and methods for selecting sampling sites are not specified.

**Response:** The sample sizes were based on practical considerations in designing a screening level assessment and were not designed to achieve a specified level of statistical significance. The proposed collection of soil and drinking water samples from five categories of agricultural fields was based on feedback during dialogue sessions and finding differences between categories is not the main goal. We are conducting a screening level assessment of levels in agricultural fields and, though we agree that 100 samples is a small number to assess such a large area of land, we are operating with practical limitations. Note that the exact locations of the samples will be determined only after further discussions with dialogue parties.

Regarding the collection of samples from communities, we propose to collect soil and water samples from 100 homes in five different communities. Two of the five communities are populated by sugar cane workers and so the design is unbalanced

as suggested by Dr. Baker. Given that we do not know whether exposure to metals is a problem in the region, the analysis of 100 soil samples and 100 drinking water samples should be sufficient to indicate whether the metals hypothesis is worthy of further exploration.

c. The data analysis methods and strategy should be revised to reflect the screening-level nature of the work. For example, the statistical data analysis methods mentioned are overkill for a screening-level analysis. The study is likely to have low statistical power. Also, use of US EPA risk assessment guidance is not valid given the screening nature of the data.

**Response:** As mentioned above, the categories allow for comparisons with sample sizes greater than 20 (that is, 40 sugar cane workers vs. 60 other workers), and as a screening exercise, our primary goal is to characterize levels and screen for potentially large differences. We also agree that the study is not designed to correlate contaminant levels with CKD prevalence precisely, and so only relative comparisons are planned. We agree that using the US EPA exposure factors for the US general population would be inappropriate. We will instead select exposure factors that are consistent with the frequency and intensity of exposures experienced by agricultural workers in Nicaragua.

#### 4. Biological Monitoring:

a. Analyzing for metals alone is not useful because metals have short half-lives in blood and urine.

**Response:** There are advantages and disadvantages to both environmental and biological sampling and we view them as complementary activities. The half-lives of metals are longer in the environment and environmental samples provide information about whether and how the exposure is occurring. However, biomarker levels integrate exposure across exposure routes and pathways and could potentially yield information that would be missed if we relied on environmental samples alone. Even though half lives are shorter in blood and urine, this is a limitation if exposures are occurring during sporadic short-term activities. It seems more likely that exposures are the result of fairly routine activities such that, on average, we could expect to observe differences in biomarker levels among different populations.

b. Using an XRF for bone lead may be more appropriate for analyzing metals.

**Response:** We agree that XRF measurements for bone lead may be useful if lead turns out to be a high priority hypothesis.

#### 5. Work Observations:

a. Heat stress in agricultural workers has been studied and it is not clear why this situation is sufficiently unique to require new studies.

**Response:** Although heat stress fatalities in farm workers have been well-reported (CDC. Heat-related deaths among crop workers –United States, 1992-2006, MMWR 57:649-653, 2008.), we are unaware of studies that examine pathophysiologic events such as dehydration/volume depletion, and muscle and kidney damage as a consequence of working in a hot environment. Our proposed studies will assess the

incidence of such abnormalities and provide a potential link to the development or exacerbation of CKD.

b. Blood serum and urine markers may not be sensitive enough in a cross-shift study to give insights regarding the volume-depletion/muscle damage hypothesis. This activity needs to be better described to justify the cost.

**Response:** Because there is evidence that occupations associated with heavy manual labor in a hot environment (sugar cane workers and miners) have the highest prevalence of CKD, we thought it was important to explore the potential pathophysiologic events occurring under these conditions. As such, the work observation is designed to fulfill this goal. Myoglobin (in serum and urine) and creatine kinase are very sensitive indicators of muscle damage and have been shown to become abnormal with a single moderate exercise exposure in untrained volunteers. If muscle damage is occurring, we believe that these measurements are sufficiently sensitive to detect it. Similarly, changes in weight, blood pressure, and heart rate are reasonable markers of severe volume depletion, although these signs are not sensitive enough to detect mild volume depletion. We agree that our proposed measurements are unlikely to detect kidney damage, although the use of tubular protein measurements has not been evaluated under these circumstances. Because the analysis of these urine markers may be expensive, a nested case-control design may be most efficient for conducting these analyses with cases representing workers with muscle enzyme abnormalities and controls representing workers without abnormalities.

6. Cohort Study:

a. The precise method for cohort sub-sampling needs to be developed as understanding of the entire records database develops. For example, the report proposes sub-sampling the records of 4,000 out of 20,000 workers but the statistical power/precision of this is unclear.

**Response:** We agree and will refine the sampling methods and sample size, as needed, following the pilot phase. We also appreciate Dr. Baker's suggestion that a nested case-control study may be appropriate and will finalize the cohort study design after the pilot phase and input from the Scientific Advisory Board.

7. Urinary Protein in Adolescents:

a. This activity should be considered a lower priority. The sampling approach needs to be developed in more detail to avoid bias in the included population.

**Response:** We will consider different sampling strategies in adolescents. This activity is not scheduled to begin until August 2010.

8. Post-Mortem Renal Biopsy:

a. Autopsies and renal biopsies should be done by local professionals.

**Response:** We propose enlisting local radiologists to perform the biopsies under ultrasound guidance.

9. Key Informant Interviews:

- a. Discussion of methods is limited but this is a highly important part of the work.

**Response:** We agree that this is a highly important part of our work and have expanded our activities in this area. See more detailed response to Dr. Orozco's comments on key informant interviews.

I. **Timeline and Budget:**

1. The timeline is ambitious. Proposed scoping activities should give insights into how feasible the timeline is.

**Response:** We agree that the timeline is ambitious and that we will develop a better sense of its feasibility over the coming months.

**Dr. Desmond Williams**  
**Centers for Disease Control and Prevention**  
**Atlanta, Georgia, USA**

**I. General:**

1. The BU group should be commended for a very comprehensive review of CKD in Nicaragua. The report is well-written and logically presented.

**Response:** We thank Dr. Williams for his positive comments.

2. Suggests adding a demographer and survey expert to the team.

**Response:** Dr. Brooks has expertise in population-based survey sampling and questionnaire construction from his work experience leading the Massachusetts Behavioral Risk Factor Surveillance Study for six years. However, we are planning to consult with a survey researcher on all questionnaires which we develop.

3. Given the uncertainty regarding prior study methodology, procedures, and laboratory techniques, the hypothesis of increased occurrence/incidence of CKD in the region is not well supported. Given that this is a central hypothesis of the recommended activities, I recommend that an additional survey be conducted to determine the true state of CKD in Chinandega.

**Response:** We agree that there are weaknesses in the existing prevalence and mortality data but we believe that the data all point in the same direction. Nevertheless, while not conducted by our group, the current survey of 3,000 households in the municipality of Leon by UNAN-CIDS and the University of North Carolina, which is being conducted with a great deal of methodological rigor, meets Dr. Williams' concern for an additional high-quality and well-powered prevalence study. We plan to monitor their results closely, and will consider whether we need to modify our activities based on their findings.

**II. Background:**

1. Strike the reference that compares the situation in Sri Lanka to that in Nicaragua.

**Response:** We have changed the language comparing the situation in Sri Lanka to that in Nicaragua.

**II. Causes of CKD:**

1. Heat and dehydration can lead to high creatinine concentrations that would not necessarily indicate CKD.

**Response:** We agree that it is possible to have a rise in creatinine associated with volume depletion. Such conditions may have affected prevalence estimates for CKD, if the conditions under which blood samples were collected were not carefully controlled. However, it may be difficult to distinguish whether volume depletion has caused a rise in creatinine due to hemoconcentration or due to an actual fall in the glomerular filtration rate. When we measure creatinine levels, we plan to collect samples in the morning prior to the beginning of the work day to limit the influence of heat and dehydration.

2. The contribution of diabetes and hypertension should not be discounted given that together they are the major cause of CKD in the world and are likely present in Nicaragua.

**Response:** We agree the contribution of diabetes and hypertension should not be discounted and are planning to collect data on these chronic diseases in all of our proposed studies and to review medical records to determine whether we find an association between diabetes and/or pre-existing hypertension and CKD.

3. The relation between the indigenous character of people in the region and CKD should be investigated further.

**Response:** We agree that the indigenous character of the people could be important and so we will collect questionnaire data on the “ethnicity” of all study participants, though we do not think this is likely to yield a great deal of useful information. We are also exploring the possibility of collecting buccal cells for genetic analyses. Given the lack of a clear candidate genetic polymorphism coupled with the cost and high false-positive rates associated with genome-wide association studies, there does not seem a clear way to make much progress on this question at a reasonable cost at this time.

#### **I. Recommended activities:**

1. The statistical power of each sampling activity needs to be described.

**Response:** The sample sizes were estimated based on practical considerations in designing a screening level assessment and so they were not designed to achieve a specified level of statistical significance. We propose to collect samples from 100 males and 100 females who live in the same homes in five different communities. Two of the five regions include sugarcane workers (40 males and females) and so we have over-sampled these workers. Given that we do not yet know whether exposure to metals is a problem, the analysis of 200 samples will be sufficient to indicate whether the metals hypothesis is worthy of further exploration.

**Dr. Miguel Orozco**  
**UNAN-CIES, Managua, Nicaragua**

1. The approach is comprehensive and commendable.

**Response:** We thank Dr. Orozco for his positive comments.

2. Suggest key informant interviews to better understand habits and beliefs surrounding traditional medicines, hygiene, dehydration, and diet.

**Response:** The suggestion that we consider qualitative approaches is very much appreciated by our team, members of which have experience with qualitative research methods. Qualitative methods are usually appreciated by epidemiologists for their ability to suggest hypotheses in the early stages of research. For this reason, we propose to conduct “key informant interviews” early in our research. What we learn during these interviews may suggest new or more focused research directions and hypothesis testing. However, we understand that Dr. Orozco is suggesting that we also consider using qualitative research methods to follow-up some of our observations during our other data collection and analysis activities. For example, if we discover during environmental sampling that people who live in a certain area have beliefs about drinking water sources or food varieties, we should consider a qualitative follow-up with a sample of residents to learn more about their opinions and beliefs. We will seriously consider how qualitative methods may further our knowledge of CKD throughout our research as well as how these approaches may shape our research direction.

3. More emphasis on evaluating the potential role of traditional medicine and therapies.

**Response:** During the “key informant interviews” we plan to interview several people about traditional medicine and therapies. Perhaps Dr. Orozco can provide us with the names of some knowledgeable individuals.

4. Suggests incorporating drinking water analysis into a surveillance system that would give people continual information.

**Response:** We would like to gather more information on drinking water issues in this region of Nicaragua and are planning to invite representatives of ENCAL, CIRA-UNAN, and other relevant agencies to our stakeholders’ meeting in December.

5. Suggests determining the experience with CKD of other sugar cane growing areas such as El Salvador, Honduras, Guatemala, and Cuba. This could be done in coordination with PAHO.

**Response:** We think that this is an excellent idea and hope that Dr. Orozco and others can help us develop these collaborations.

6. Suggests a list of potential local partners and stakeholders.

**Response:** We thank Dr. Orozco for this helpful list of potential partners and collaborators. We will pursue these contacts.