



**Executive Summary
Scoping Study
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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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.

Areas of potential investigation	Implications for Action
<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</p>	<p>Rhabdomyolysis is a recognized, if rare, cause of acute kidney injury and may occur with exertional heat stroke. With</p>

<p>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>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><i>Systemic Infections:</i></p> <p><i>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.</i></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 failure in the setting of severe volume depletion or other nephrotoxins.</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><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</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</p>

<p>environmental temperatures. Therefore, there may well be an increased risk for kidney stones and possibly related CKD in Nicaragua.</p>	<p>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 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>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 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</p>	<p>The prevalence of UTIs could be addressed in a study of children where there is less recall bias; while this would</p>

<p>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>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

agricultural chemicals. 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 agricultural chemicals. 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 of 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 agricultural chemicals 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 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 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 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:

Role	# positions
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 be 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.