Overview of DiNEH Project: History, Methods, Results

Presentation to Collaborators in U.S.-Russia Health Risk Research Dialogue

For September 25, 2014 Webinar
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Background: A boy watches crews remove radium- and uranium-contaminated soils from around his home in the Red Water Pond Road Community next to the Northeast Church Rock Uranium Mine on the Navajo Nation near Gallup, New Mexico, USA.

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A Message to Our Russian Colleagues

The DiNEH Project research team is comprised of scientists with the University of New Mexico Community Environmental Health Program (UNM-CEHP), UNM College of Pharmacy and Southwest Research and Information Center (SRIC), in collaboration with the people of the Eastern Agency of the Navajo Nation. We are sharing the history, methods and results of the DiNEH Project to provide you with analogous information as you design a health study that meets the needs of the people of Zakamensk, Buryatia (map at right). We believe the DiNEH Project is the most relevant study to your investigation of the effects of environmental exposures to mining wastes in Zakamensk. The UNM-SRIC team will provide details on the biomedical findings of the DiNEH Project, especially on the mechanisms of metals toxicity from exposures to mining wastes, during our face-to-face meetings in New Mexico in November 2014.
Diné Network for Environmental Health (DiNEH) Project*

**Kidney Health Project History and Purpose**

- “DiNEH” – both acronym and phonetic pronunciation of the Navajo word “Diné”, meaning “the People”
- 2000-2011; data analysis 2011-present
- Original community question: *Does ingesting uranium in unregulated water increase risk of kidney disease?*
- Study evolved to more broadly examine environmental uranium exposures and community health
- Community-based participatory research (CBPR) model
  - Community members help design and participate in study
  - Study builds Navajo community research capacity
  - Study conducted with respect for culture and language
- Commitment to inform policy and improve clinical care

*The DiNEH Project has been approved by the Navajo Nation Human Research Review Board and the University of New Mexico (UNM) Human Research Review Committee.*
Context of DiNEH Project: Pervasive Exposures to Uranium Sources

At least 1 uranium exposure source is present in 57 of the 110 chapters of the Navajo Nation. About 20 chapters have water sources contaminated with uranium.

Map by T. Rock, DiNEH Project

A “chapter” is the unit of local governance on the Navajo Nation.

DINEH Study Area: 11 of 20 chapters have 1 or more U exposures.
Context: Community health concerns when DiNEH Project launched in 2000

- Health disparities:
  - Diabetes: 3-5x greater than U.S. rate as a whole
  - Chronic Kidney Disease: 2.5x
  - End-Stage Renal Disease: 3x
- Early onset: teenagers on dialysis
- >30% Navajo population lacked access to regulated, community water piped into their homes
  - Routinely used unregulated wells
  - Water quality wasn’t known
- Anthropogenic and natural uranium surface exposures sources
Summary of DiNEH Project Activities

- First population-based study of Navajo environmental exposures, co-morbidities
- Navajo-speaking field staff
- 20 chapters in Eastern Agency
- 1,304 participants surveyed over 6-year period
- Median age, 51; median residency, 33 yrs

- Phase I: Completed
  - Participant surveys
  - Locations of participant homes, abandoned uranium mines
  - Water quality assessments

- Phase II – Completed blood and urine sample collection and biomarker analyses for 267 participants; laboratory and statistical analyses continuing
DiNEH Project Risk Model

Sources of inputs to estimate each participant’s total exposure

Kidney Risk Model—Structure and Multiple Inputs

Water and environmental exposures highlighted

Core Dose-Response Model

Core Exposure Assessment Model

Kidney Disease
- Self report, medical records, clinical test

Biomarkers
(SA1)
- Urine, lab analysis

Uranium exposure assessment
- Input from Core Exposure Assessment Model, Urine Confirmation

Other exposures
- Survey, GPS, Extant location

Co-morbidities
- Survey, medical records

Family history
- Survey

Tradition, culture, lifestyle
- Survey

SES factors
- Survey

Occupational Exposure
- Survey

Water Exposure
- Survey, lab analysis

Environmental Exposure
- Survey, mapping, modeling, field validation

Modifying factors


DiNEH Project Methods

Planning and Design (2002-2004)

- Broad community training, participation in study purpose and design
- Responded to community concerns about possible environmental factors in high rates of kidney disease, diabetes
- Extensive research of literature on uranium toxicity
- Study Region: 20 Communities in Eastern Navajo Agency
- Cross-sectional study design captures exposed and not-exposed individuals

Above: Eastern Navajo Health Board training, Feb. 2002; middle: water testing training, March 2003

Above: Sarah Adeky and Johnnye Lewis, Church Rock Chapter, 2007
DiNEH Project Methods (continued)

Exposure Assessment

- Consent to participate (photo)
- Survey (right)
  - Developed with Navajo community input, oversight
  - 45 questions in 4 categories: Demographics, Water Use, Environmental and Occupational Exposure, Medical, Family and Cultural History
    - Administered by trained Navajo-speaking community members in Navajo or English or both
- Medical Records Review
  - 85% agreement between participant responses and medical record data
DiNEH Project Methods

Exposure Assessment (continued)

- Environmental Sampling
  - Sampling and analysis of water from 130 different drinking water sources
  - Soil sampling in areas representative of impacted and non-impacted sites

- Geospatial Data
  - Latitude-longitude coordinates for each participant’s home
  - Locations of uranium mines and mill waste sites

DiNEH study area is ~50 miles N-S, 100 miles E-W (81 x 161 kilometers)
DiNEH Project Methods (continued)

Health Assessment

- Conducted in collaboration with Navajo Area Indian Health Service CUE-JTH program
- Statistical analyses of survey responses and geospatial data (manuscript submitted)
- Blood and urine provided by a subset of participants
- Clinical measures (e.g., blood pressure, body-mass index, hemoglobin A1C, antinuclear antibodies)
- Analyses of biomarkers of effects of exposures

Blood and urine collection, sampling processing, at Baca-Prewitt Chapter, August 2010.
Why collect blood & urine samples?

- Obtain biological confirmation of self-reported health conditions (from survey responses)
- Assess overall health of study participants by testing for clinical measures of disease
- Assess blood and urine for biomarkers of early disease
  - Biomarkers – any biological indicator of a particular disease state or stage of disease
  - Measures effects of exposures
- Investigate disease status in relation to environmental exposures
  - Hypertension (i.e., high-blood pressure)
  - Cardiovascular disease (CVD) (i.e., arteriosclerosis)
  - Kidney disease
  - Autoimmune disease
DiNEH Iterative Assessment

- Capacity Building – Multi-directional study design
- Surveys: 1,304 participants, median age 51

Medical Record Reviews

Clinical Assessments: blood and urine from 267 participants

Biomarker Analyses

Our analyses have consistently shown a relationship between uranium waste exposures and chronic disease over several iterations as more participants entered the study.
Navajo health disparities confirmed

Prevalences of Self-reported Chronic Diseases Among DiNEH Project Participants (N=1,304) compared with U.S. Prevalence Rates

*DiNEH cancer prevalence based on 1,011 respondents

Note: U.S. prevalence rates from on-line reports of CDC, American Heart Association, and various professional organizations. Prevalence of heart attacks and prevalence of “no disease” in U.S. not reported by those agencies.
Results of Bayesian Modeling of survey responses and geospatial data:
Active-mining era exposures (workers and families) increased risk of kidney disease.

Active-mining related exposures were estimated from self-reported survey data:
A: Washed the clothes of a uranium worker (22%)
B: Worked in a uranium mine (10%)
C: Lived in a mining camp (4%)
D: Worked in a uranium mill (2%)
E: Worked on a uranium mine or mill reclamation or hauled uranium ore or tailings in a pickup truck (2%)

Note: Many workers have already died from lung cancer; cohort had more family members than workers.
DiNEH Results:

Ongoing environmental legacy exposures → *increased risk* for hypertension, autoimmune disease, immune dysfunction

Exposures estimated from **self-reported activities***

A: Used materials from abandoned uranium mine, mill (17%)
B: Herded livestock next to uranium mine, mill or waste dump (13%)
C: Drunk or contacted uranium mine waste water (13%)
D: Played on a uranium tailings pile or waste dump (13%)
E: Played outdoors near a uranium mine, mill, or waste dump (12%)
F: Sheltered livestock in an abandoned uranium mine (2%)

*Note: Median length of residence in current homes was 33 years
DiNEH Results:

Increased risk of high blood pressure

All other risks being equal, we found two significant factors:

- **Proximity** to abandoned uranium mine and mill wastes +
- Number of self-reported activities that bring people near or in contact with uranium wastes

*These maps show a “dose-response relationship” for HBP*
Environmental legacy exposures increase the likelihood **autoimmune disease**

- Proximity to uranium wastes *significant predictor* +
- Risk of *autoimmune disease* (self-reported) increases with increasing number of exposure activities
  - Indicates a *dose-response relationship* – the greater the exposure, the greater the risk of disease

1 Exposure Reported  ➔  2 Exposures Reported
What is causing these effects?

- Living next to mine wastes?
- Breathing contaminated dusts from mine sites?
- Surface water runoff from mine sites?
- Drinking from contaminated wells?
- Eating meat from livestock that grazed on mine sites?
- Radiation from uranium mine wastes? Or heavy metals?

We think it is a combination of all of these pathways and routes of exposure, and that’s why we are interested in the mechanisms of toxicity.
More UNM-SRIC Research Team Results
We will discuss these during our face-to-face dialogue in Nov. 2014

Environmental Assessments
- Water sampling results for 130 water sources tested in DiNEH study area
- Soil sampling for uranium, other metals
- Mine-waste characterizations for metals, particle sizes
- Uranium solubility and transport in aqueous systems
- Risk mapping and risk communication

Biological Assessments
- Recent cardiovascular and immunological assays
- Zinc intervention to stop or reverse arsenic and uranium damage to DNA
- Investigation of inhalation risks of metals mixtures in mine wastes using animal models
- Metals occurrences in blood and urine of pregnant mothers and newborn babies in Navajo Birth Cohort Study
**Water is the pathway, ingestion the exposure route**

- **Target Organ:** Where a contaminant ends up in the body; e.g., bone, kidney, lung

**Exposure Pathways:** environmental, outside the body

- Air, water, plants, animals, humans
  - (can be very simple or quite complex)

**Exposure Routes:** inside the body

- How contaminants enter the body

**Sources:** Potentially harmful contaminants in the environment

**Exposure Pathways:** environmental, outside the body

**Exposure Routes:** inside the body

- Inhalation (Breathing)
- Ingestion (Eating, Drinking)
- Absorption (Skin Contact)

**Circulation:**
- Transplacental transfer?
- Epigenetic changes?
**Pathways:**

Uranium mine wastes contribute heavy metals, radionuclides to soils and groundwater

<table>
<thead>
<tr>
<th>Element</th>
<th>NECR Mine wastes, maximum values</th>
<th>NECR Mine wastes, average values</th>
<th>Non-impacted (normal) soils, median values</th>
</tr>
</thead>
<tbody>
<tr>
<td>U (ppm)</td>
<td>3,970</td>
<td>79.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Ra-226 (pCi/g)</td>
<td>875</td>
<td>30.6</td>
<td>1</td>
</tr>
<tr>
<td>As (ppm)</td>
<td>14.9</td>
<td>4.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Uranium mine wastes contain

(1) all the radioactive elements that decay from natural uranium, or U-238, such as Ra-226; and

(2) uranium, arsenic and a wide range of heavy metals, including iron, lead, nickel, vanadium
Mechanisms of toxicity

Uranium (U) has both chemical and radiological toxicity

- **Chemical effects:**
  - Readily forms compounds with negatively charged ions, like oxygen (O\textsubscript{2}) and carbonate (CO\textsubscript{3})
  - **Target organ is the kidney**
  - U ion degrades, kills cells in proximal, distal tubules
  - May have estrogenic effects

- **Radiation effects:**
  - U decays to radium-226, which deposits in the bones, causing bone cancers
  - U decays to radon and its “daughters”, which cause lung cancer

- Based on hundreds of studies in laboratory animals, humans and uranium workers over past 100+ years
What do metals in uranium mine wastes have to do with Cardiovascular Disease (CVD) and Diabetes?

CVD (especially hypertension)
- Prevalence increasing in Navajo community
- May be promoted or worsened by environmental exposure to heavy metal contaminants

Diabetes
- Diabetes — risk factor for CVD
- Prevalence increasing in Navajo community
- Does diabetes increase susceptibility to metals?
- *Mine wastes are mixtures of many metals*

DiNEH prevalences (chart)
- Differences between self-reports and blood & urine confirmation may reflect self-selection bias

### DiNEH self-reported and biologically confirmed prevalence rates compared with U.S. Rates

<table>
<thead>
<tr>
<th>Condition</th>
<th>DiNEH self-reported (N=1304)</th>
<th>DiNEH B&amp;U (N=252)</th>
<th>US Prevalence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes II</td>
<td>25.1%</td>
<td>26.2%</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>29.6%</td>
<td>35.9%</td>
<td>38.1%</td>
</tr>
<tr>
<td>Heart Disease</td>
<td>25.4%</td>
<td>5.4%</td>
<td>6.0%</td>
</tr>
<tr>
<td>Stroke</td>
<td>9.3%</td>
<td>5.2%</td>
<td></td>
</tr>
</tbody>
</table>

*DiNEH prevalences (chart) vs. U.S. rates.*
Cardiovascular Disease: Atherosclerosis

LDL ("Bad") Cholesterol

 Metals

 oxLDL marker for CVD

• Vessel Dysfunction
  • Inflammation
    • Immune Responses
    • Oxidative Stress
**DiNEH Project Results:**

**Arsenic and uranium in water associated with cardiovascular inflammation**

<table>
<thead>
<tr>
<th>Total number different water sources</th>
<th>376</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Sources Exceeding at least 1 MCL* at least 1 time</td>
<td>103</td>
<td>27.4%</td>
</tr>
<tr>
<td>Arsenic</td>
<td>65</td>
<td>17.3%</td>
</tr>
<tr>
<td>Uranium</td>
<td>38</td>
<td>10.1%</td>
</tr>
<tr>
<td>Gross alpha radioactivity</td>
<td>9</td>
<td>2.4%</td>
</tr>
<tr>
<td>Selenium</td>
<td>8</td>
<td>2.1%</td>
</tr>
<tr>
<td>Nitrate</td>
<td>7</td>
<td>1.9%</td>
</tr>
<tr>
<td>Radium-226+228</td>
<td>7</td>
<td>1.9%</td>
</tr>
<tr>
<td>Fluoride</td>
<td>6</td>
<td>1.6%</td>
</tr>
<tr>
<td>Thallium</td>
<td>5</td>
<td>1.3%</td>
</tr>
<tr>
<td>Lead</td>
<td>3</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

- Arsenic (As) and uranium (U) — two most frequent contaminants in unregulated water sources on Navajo Nation (see table at left)
- Unregulated water sources contributed *vast majority* of As and U intake among DiNEH participants
- Annual arsenic intake *associated* with oxLDL* (p=0.04) (see chart below left)
- Uranium intake weakly associated with oxLDL
- Age, occupational exposures (M), distance to and contacts with mine wastes (E) also *significant predictors* of oxLDL

<table>
<thead>
<tr>
<th>(Intercept)</th>
<th>3.9835</th>
<th>0.0951</th>
<th>41.8834</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.0032</td>
<td>0.0016</td>
<td>-2.0240</td>
</tr>
<tr>
<td>M</td>
<td>0.0788</td>
<td>0.0268</td>
<td>2.9409</td>
</tr>
<tr>
<td>E</td>
<td>-0.4609</td>
<td>0.1725</td>
<td>-2.6718</td>
</tr>
<tr>
<td>ahigh</td>
<td>0.2092</td>
<td>0.0989</td>
<td>2.1141</td>
</tr>
</tbody>
</table>

*oxLDL = oxidized low-density lipoprotein*
Arsenic intake from contaminated drinking water may influence oxidative modifications of lipoproteins and promote cardiovascular disease in Navajo populations.

Uranium intake associated with oxLDL, a biomarker of CVD, *only* when modeled as a continuous numerical value, and not associated when modeled as a binary variable (i.e., high v. low intake).

Detecting a health effect from uranium intake limited by:
- Most of the sampled unregulated water sources, and nearly all of the regulated waters, had very low levels of uranium, and
- Only a few participants drank from the most contaminated wells.

*Source: Harmon et al., Environmental Predictors of Oxidized LDL Cholesterol (oxLDL) in Navajo Populations Exposed to Uranium-Contaminated Mining Sites. Society of Toxicologists Annual and Mountain Region meetings, 2013.*
DiNEH Project Results:

**Immune system and autoimmunity**

- Immune system — human body’s defense system
- Processes work to ensure survival of the individual and reinstate good health conditions after infection
- Autoimmune diseases — conditions resulted in disturbance of healthy immune function, hyperactivity and overproduction of immune activation
- Studies of human immune response among DiNEH participants:
  - Characterize immune cells
  - Measure inflammation, cytokine production
  - Describe autoimmune processes, autoantibody production
  - Early markers, showing alterations in immune cell distribution and activity
  - Immune biomarker analyses for 69 DiNEH participants (out of 267 who gave blood and urine samples)
DiNEH Project Results:

Flow cytometry measurements

- Lymphocyte subpopulations from whole blood samples
- Becton Dickinson Simultest IMK Plus lymphocyte kit was used
- 6 cell populations were measured:
  - T cells (CD3+), T helpers (CD4+), T suppressors (CD8+);
  - B cells (CD19+)
  - HLA-DR+ cell activation in T cells
  - B cells and other cell types; NK cells (CD3-/CD16+/CD56+)
DiNEH Project flow cytometry results I

Immune impairment associated with U exposure

CD3+ T cells (in %) among DiNEH participants

\[ y = 25.601x + 44.298 \]

\[ R^2 = 0.0446 \]

CD19+ B cells (in %) among DiNEH participants

\[ y = 16.366x + 0.8651 \]

\[ R^2 = 0.1538 \]
In healthy immune system, T cells and B cells act together, in the same direction.

Graph shows increased percentage of activated T cells, decreased percentage of activated B cells.

This decoupling of T cell and B cell activities suggests altered immune response among this subset of participants exposed to uranium wastes.

Can lead to lower production of protective antibodies.
More UNM-SRIC Research Team Results

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