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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- Curtis Miller, Ph.D., UNM-CEHP (biostatistician)
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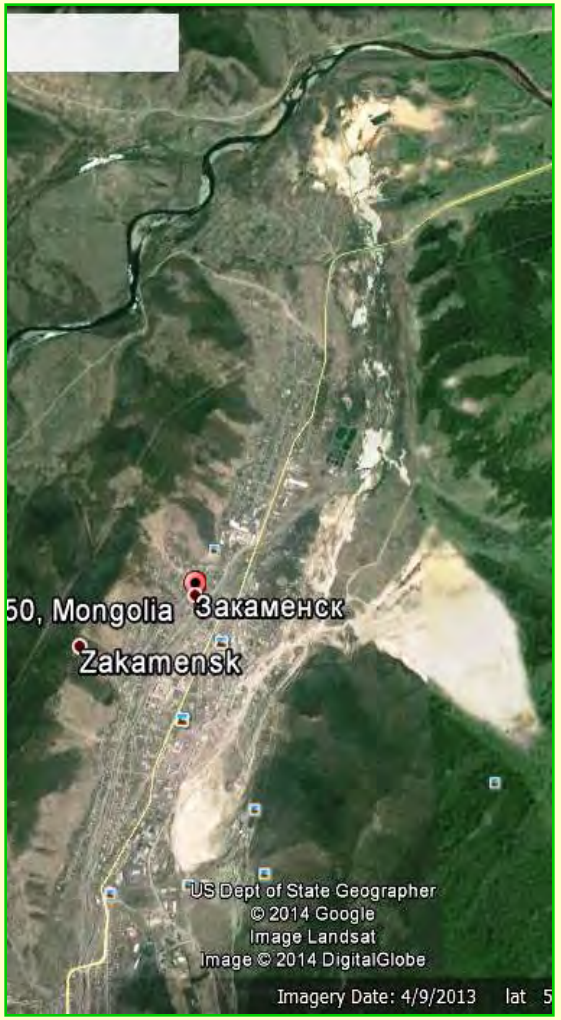
Background: A boy watches crews remove radium- and uranium-contaminated soils from around his home in the Red Water Pond Road Community next to the Northeast Church Rock Uranium Mine on the Navajo Nation near Gallup, New Mexico, USA.

10.09.2009

Funded by NIEHS, P30 ES-012072, R25 ES013208, & R01 ES014565

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

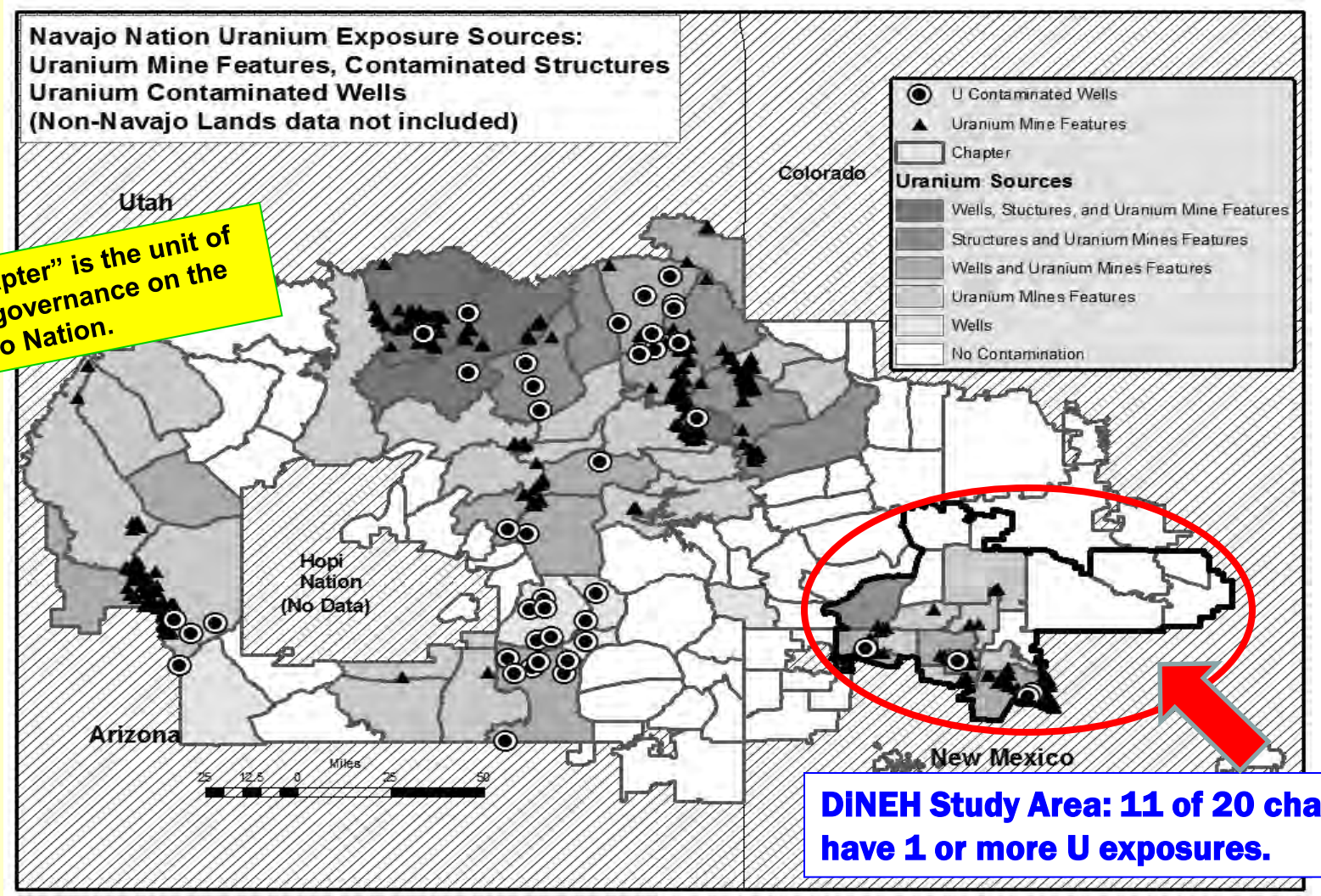
- "DiNEH" – both acronym and phonetic pronunciation of the Navajo word "*Diné*", meaning "the People"
- 2000-2011; data analysis 2011-present
- Original community question: ***Does ingesting uranium in unregulated water increase risk of kidney disease?***
- Study evolved to more broadly examine environmental 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.



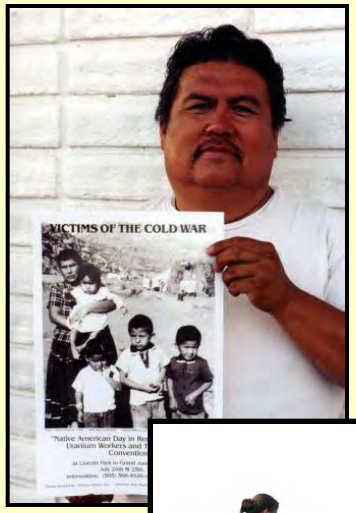
A "chapter" is the unit of local governance on the Navajo Nation.

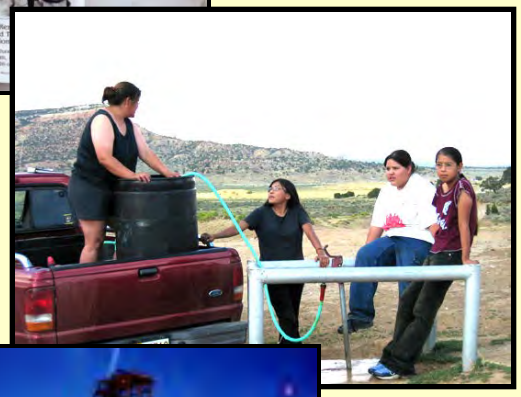
DINEH Study Area: 11 of 20 chapters have 1 or more U exposures.

Map by T. Rock, DiNEH Project

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





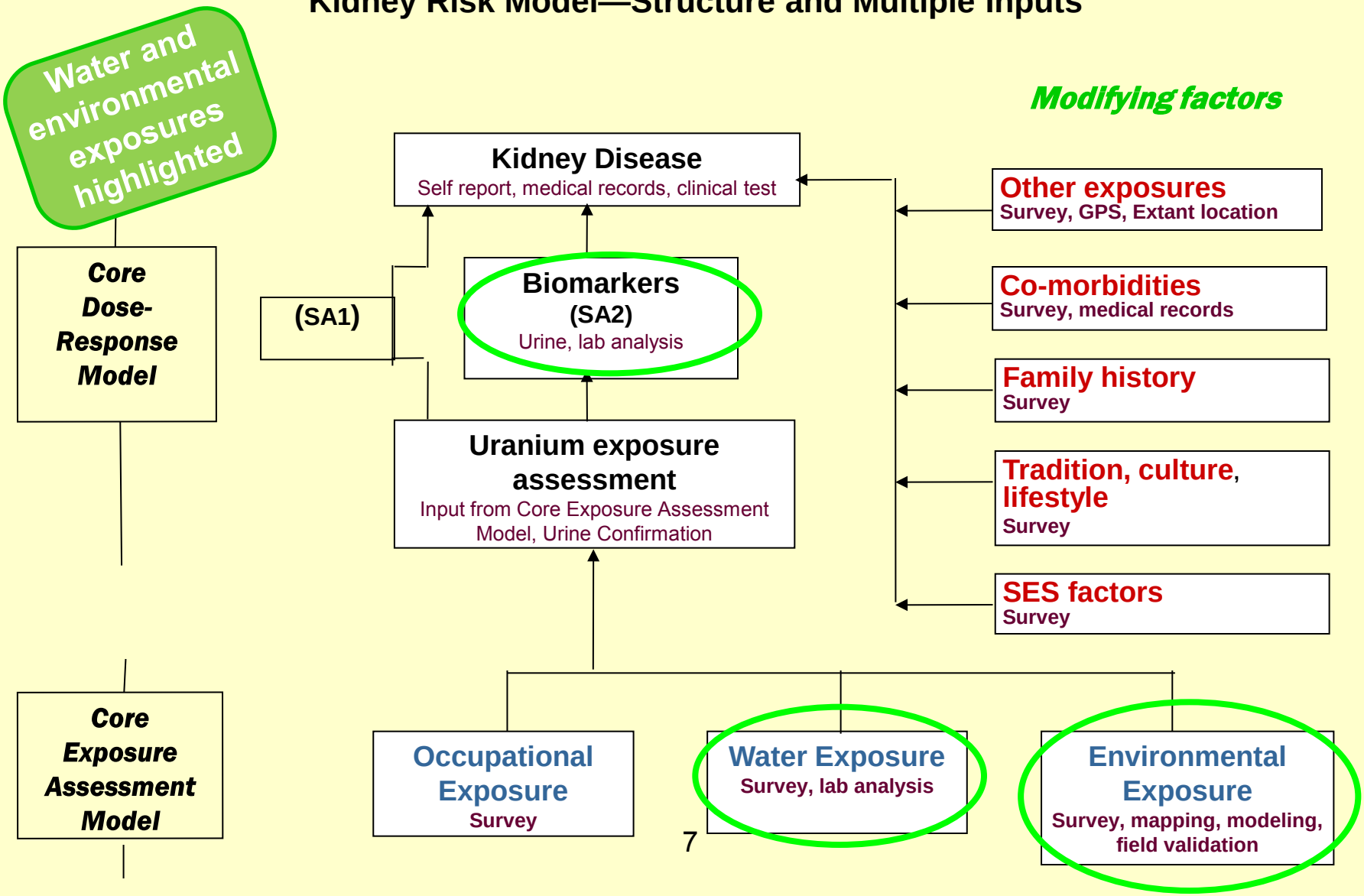
Summary of DiNEH Project Activities

- 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: <u>Completed</u>
  - 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



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

Participant Number:_____ Revision No. 11, 2/15/08

Diné Network for Environmental Health (DiNEH) Project
Water and Land Use, Environmental and Health Survey

[INSTRUCTION TO INTERVIEWER — PLEASE READ THIS INTRODUCTION AS WRITTEN BEFORE OBTAINING CONSENT OR ADMINISTERING THIS SURVEY.]

INTRODUCTION: Greetings. My name is _____ and my partner here is _____. We are conducting a survey of how people in the Eastern Agency use water they haul from unregulated water sources, such as windmills and springs. This survey is part of a study called the Diné Network for Environmental Health Project, or DiNEH Project. We are testing water from unregulated water sources, estimating how people are exposed to uranium and other contaminants that might be in the water and in their communities. We are working with 20 chapters in the Eastern Agency to identify safe and unsafe drinking water sources.

The DiNEH Project is supported by all 20 Chapters in the study area and is being conducted by the Eastern Navajo Health Board, the Crownpoint Hospital, Southwest Research and Information Center, and the University of New Mexico. The project is sponsored by the National Institutes of Health. We also have support from Navajo Nation President Joe Shirley, Jr., and approval by the Navajo Nation Human Research Review Board, or "Navajo IRB". Here is brochure that describes the DiNEH Project. Feel free to take a few minutes to read it.

This survey is purely voluntary and will take about 1 hour. And if you agree to be interviewed, we will pay you $10 voucher for goods, with a limit of 2 vouchers per household. OK, would you like to participate in this survey?
[If answer is "No", thank the person for their time and tell them they may keep the brochure. If the answer is "Yes," ask the following question:]

Do you haul water for yourself or someone else? ❑ Self ❑ Someone else
[If the answer is yes or no continue with the survey] **❑ Self & someone else ❑ Refused**

Have you been interviewed for this survey before? ❑Yes ❑No
[If 'yes', stop here and thank them for their time; if "No," proceed to the next question.]

What Chapter do you live in? _____
[If the person does NOT live in one of the 20 Chapters in the Study Area, tell them they are not eligible to participate in the study and thank them for their time. If the person lives in one of the Chapters in the Study Area, proceed.]

Would you like to be interviewed in the Navajo or English language?
❑ Navajo ❑ English ❑ Combination of both

I am going to read two documents, called "Consent to Participate in Research" and a "HIPAA" form.
[Read the Consent Form. Make sure the participant initials each page and obtain participant's signature on the form before proceeding. Hand the participant a blank copy of the Consent Form after he or she has signed the original. You, the Interviewer, will keep the original signed consent form. Make sure the HIPAA and release forms are signed also.]

Was the **"Consent to Participate in Research"** *read in* **❑ English ❑ Navajo ❑ Combination of both**

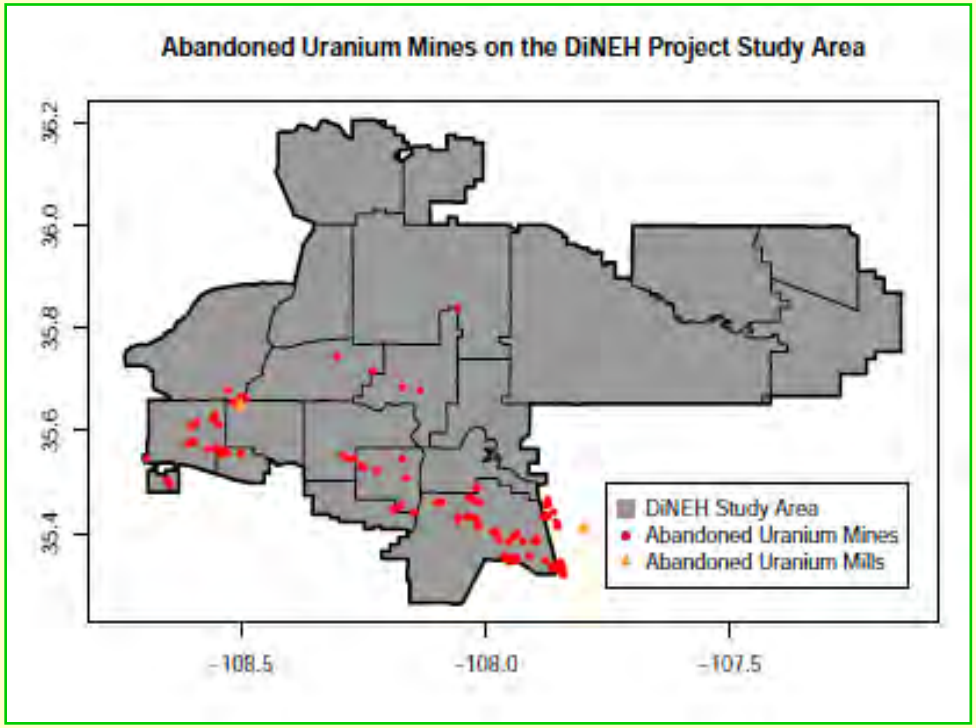
DiNEH Project Methods

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

Uranium screening clinic at Iyanbito Chapter, April 5, 2011

DiNEH Iterative Assessment

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

Medical Record Reviews

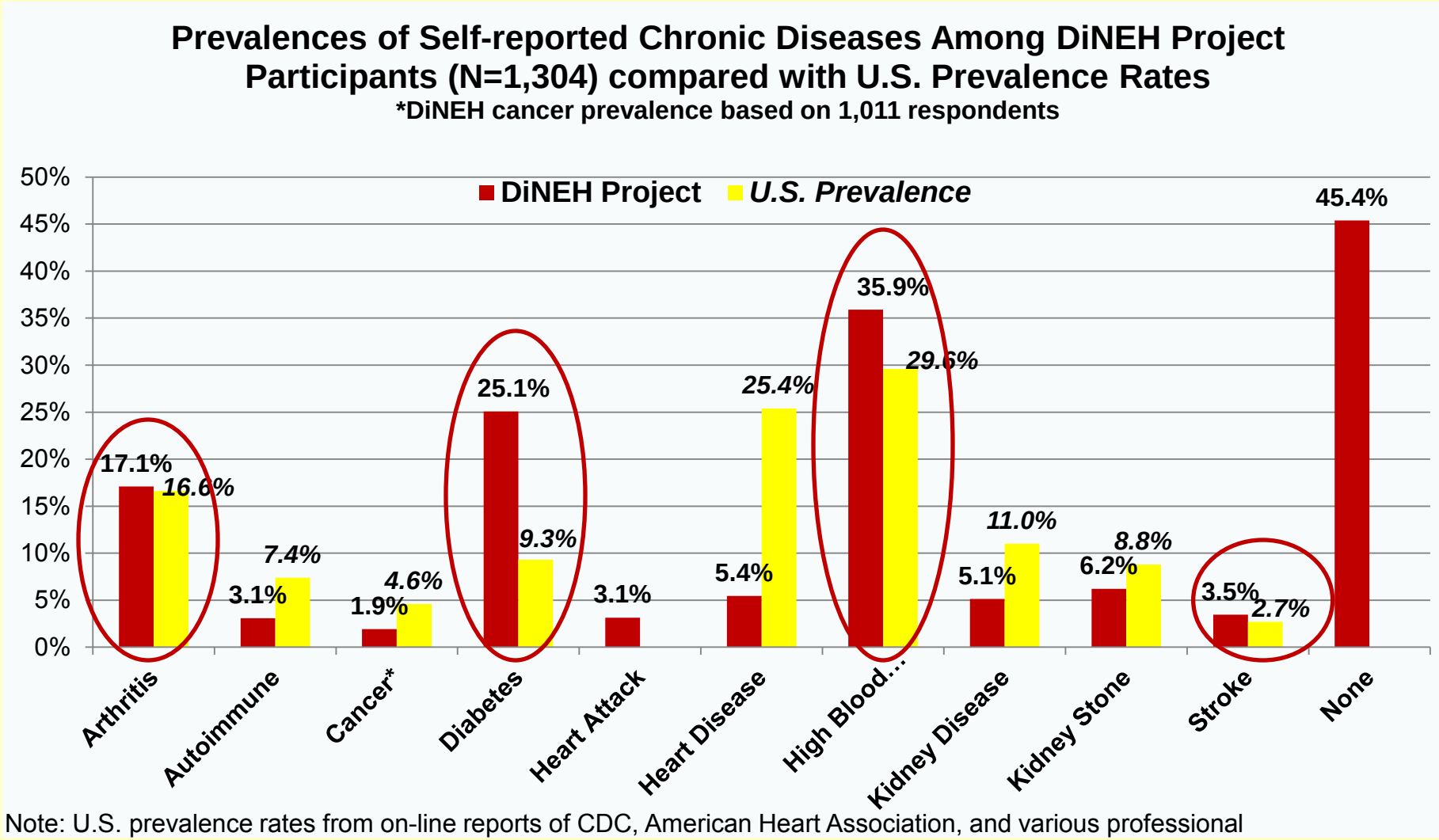
Clinical Assessments: blood and urine from 267 participants

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.

DiNEH Results:

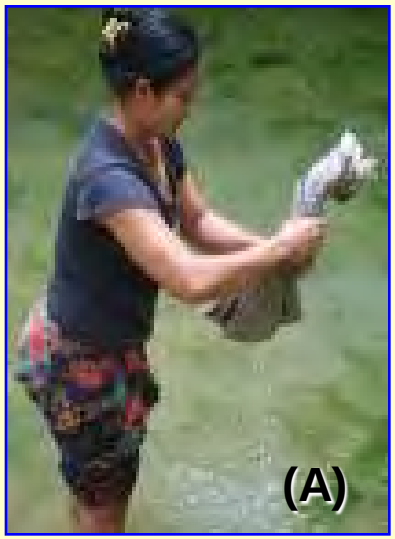
Navajo health disparities confirmed

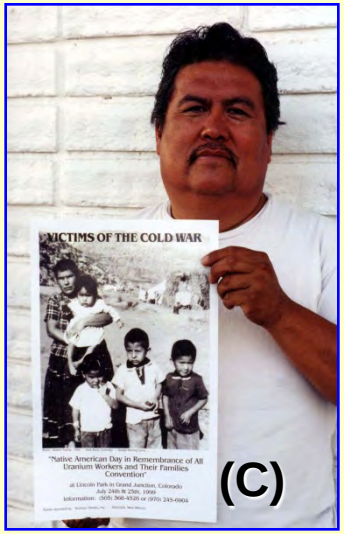


Note: U.S. prevalence rates from on-line reports of CDC, American Heart Association, and various professional organizations. Prevalence of heart attacks and prevalence of “no disease” in U.S. not reported by those agencies.

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 <u>self-reported survey data</u>

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 <u>self-reported activities*</u>:

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 <u>33 years</u>*

DiNEH Results:

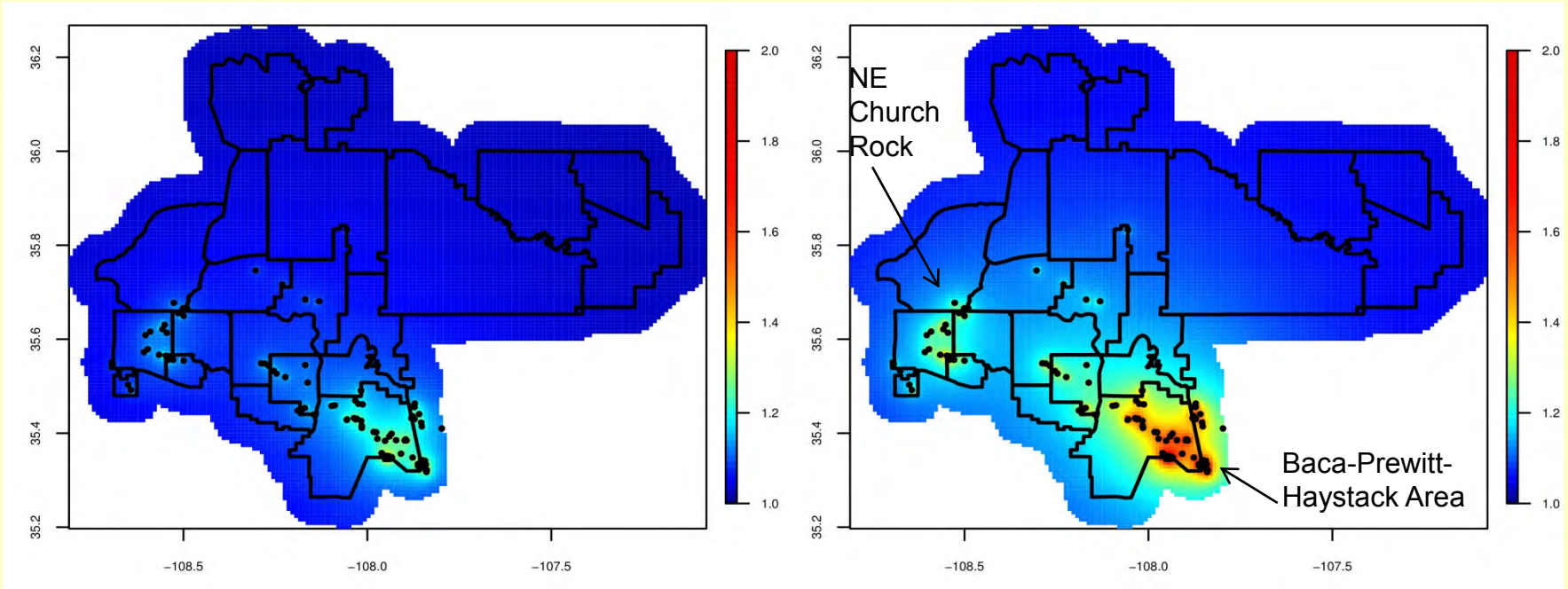
Increased risk of high blood pressure

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

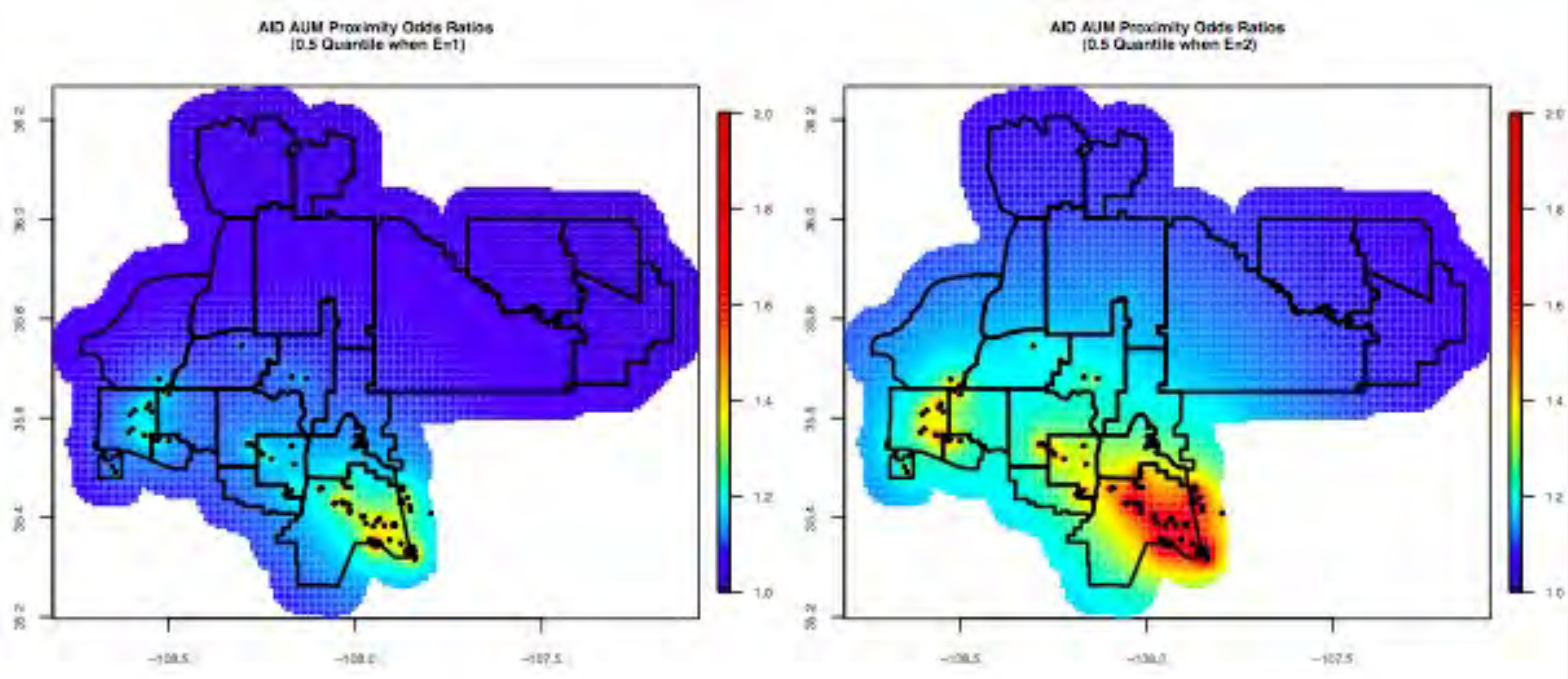
1 Exposure Reported → **2 Exposures Reported**



Environmental legacy exposures increase the likelihood *autoimmune disease*

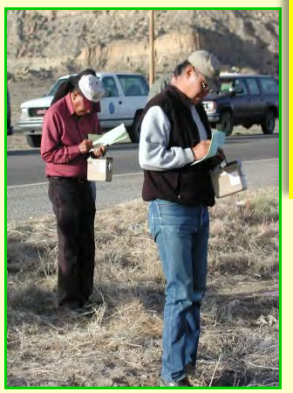
- Proximity to uranium wastes *significant predictor* +
- Risk of *autoimmune disease* (self-reported) increases with increasing number of exposure activities
  - Indicates a *dose-response relationship* – the greater the exposure, the greater the risk of disease

1 Exposure Reported → **2 Exposures Reported**

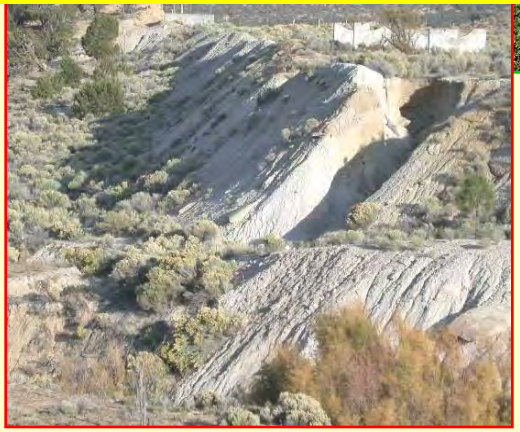


What is causing these effects?

We think it is a <u>combination</u> of all of these pathways and routes of exposure, and that's why we are interested in the *mechanisms* of toxicity



Radiation from uranium mine wastes? Or heavy metals?



More UNM-SRIC Research Team Results

We will discuss these during our face-to-face dialogue in Nov. 2014

Environmental Assessments

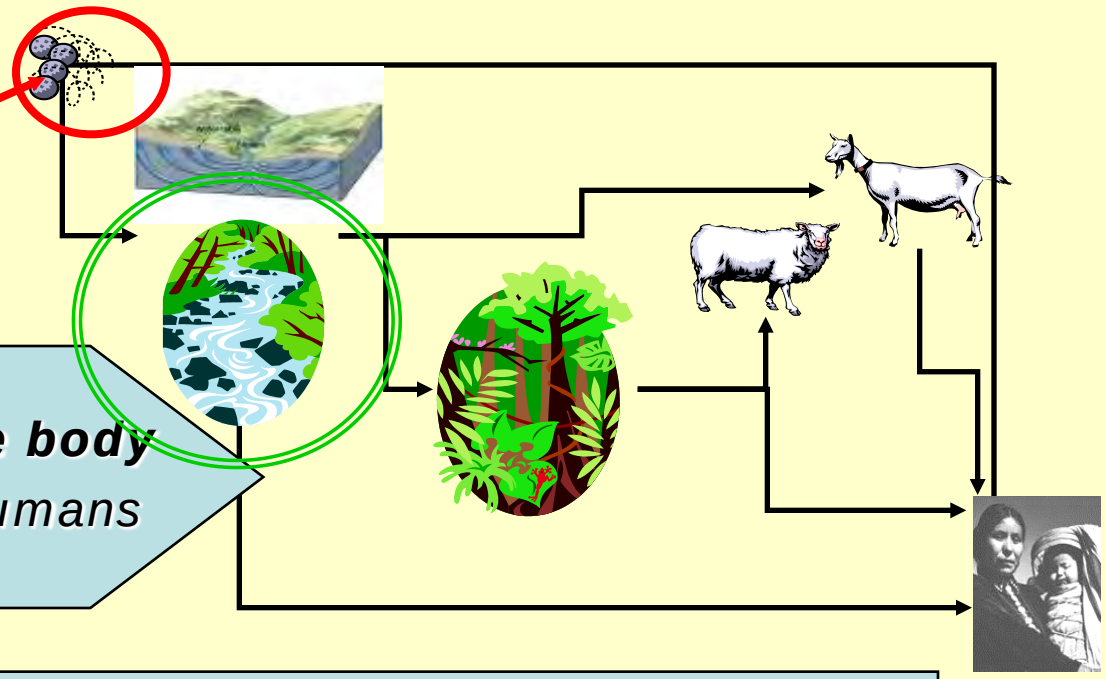
- Water sampling results for 130 water sources tested in DiNEH study area
- Soil sampling for uranium, other metals
- Mine-waste characterizations for metals, particle sizes
- Uranium solubility and transport in aqueous systems
- Risk mapping and risk communication

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

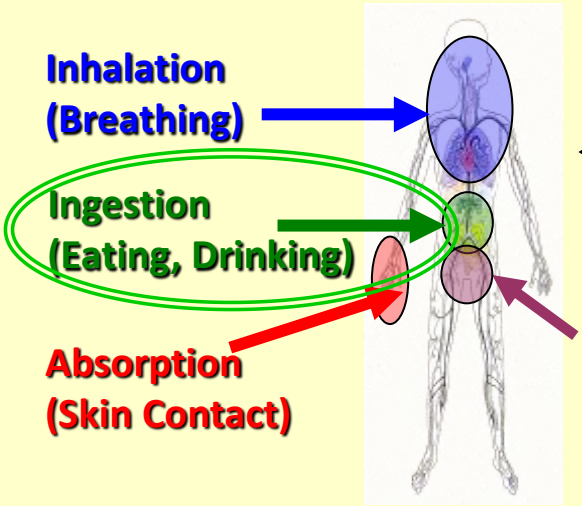
SOURCES: Potentially harmful contaminants in the environment



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

Circulation:

- **Transplacental transfer?**
- **Epigenetic changes?**

Target Organ:

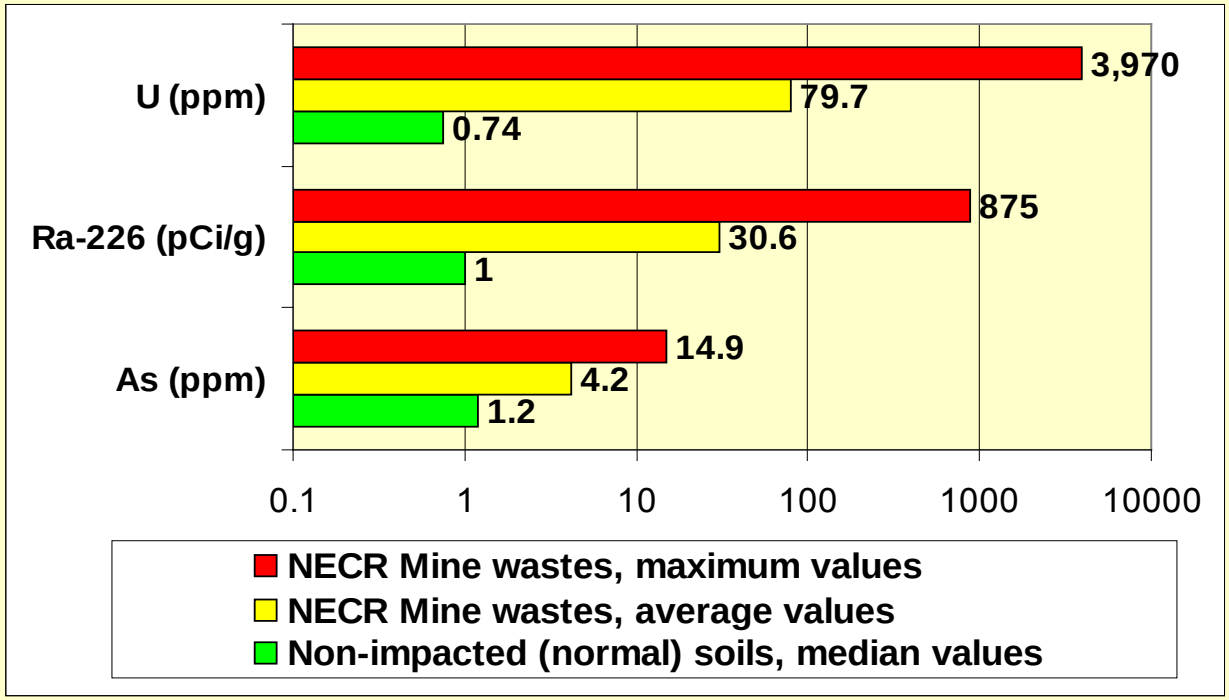
Where a contaminant ends up in the body; e.g., bone, kidney, lung

Pathways:

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

NECR Mine wastes, 2003, before partial recontoured in 2009

DiNEH staff collect background soil samples in Church Rock, 2005.



Uranium mine wastes contain

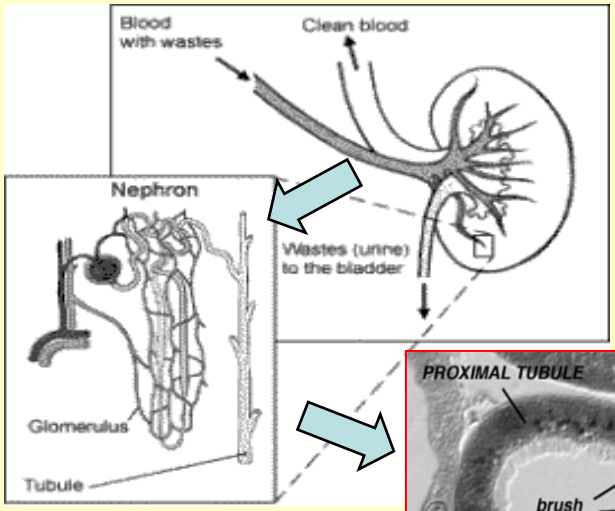
(1) all the radioactive elements that decay from natural uranium, or U-238, such as Ra-226; and

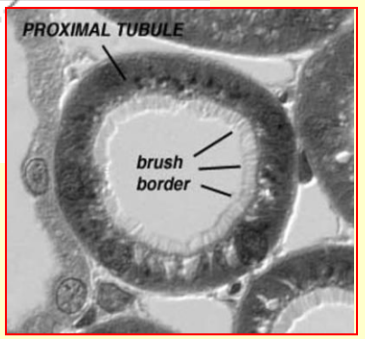
(2) uranium, arsenic and a wide range of heavy metals, including iron, lead, nickel, vanadium

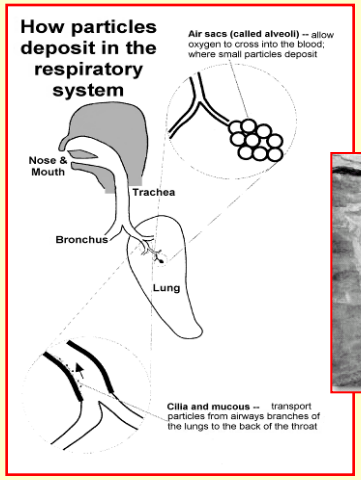
Mechanisms of toxicity

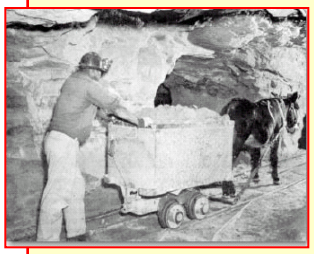
Uranium (U) has both chemical and radiological toxicity

- **Chemical effects:**
  - Readily forms compounds with negatively charged ions, like oxygen (O_2) and carbonate (CO_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

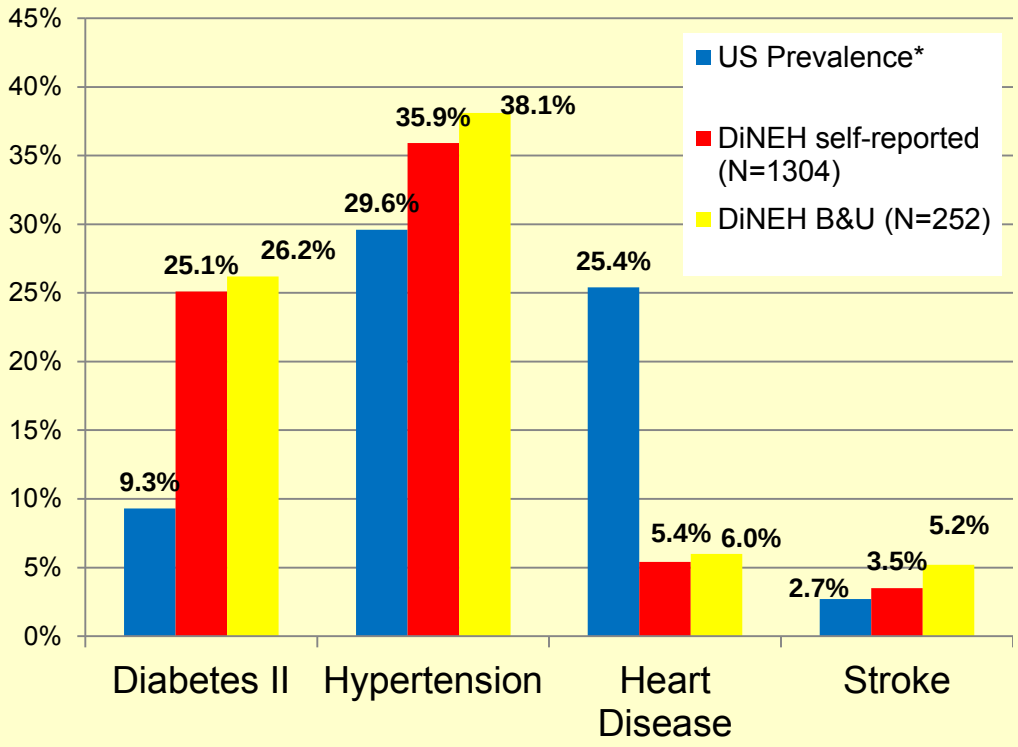
Diabetes

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

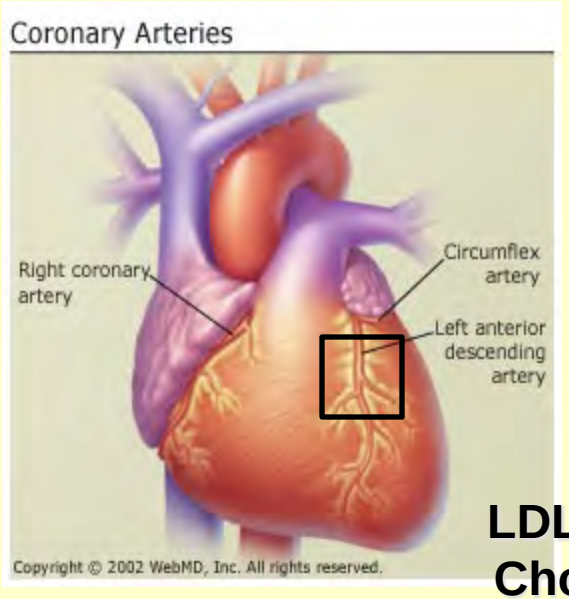
DiNEH prevalences (chart)

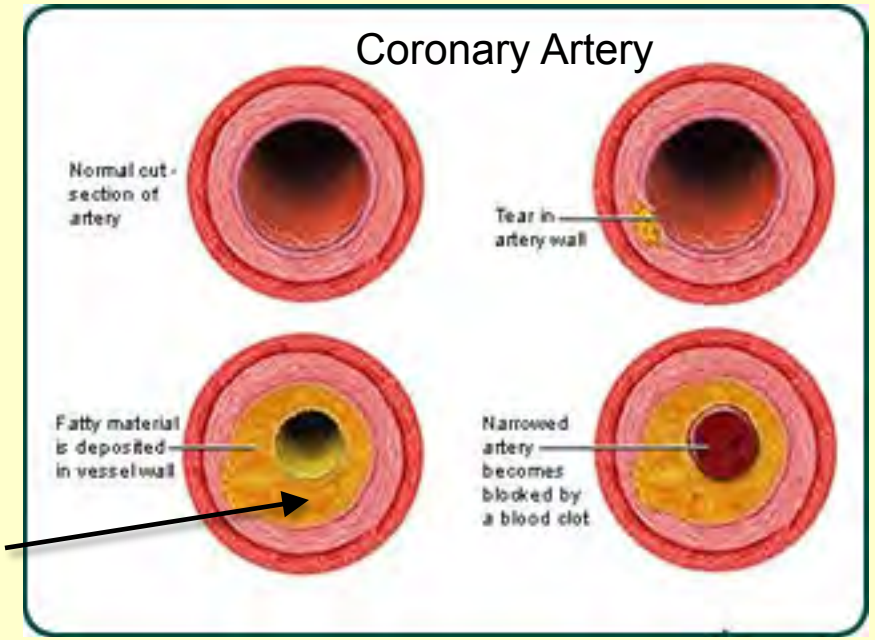
- Differences between self-reports and blood & urine confirmation may reflect self-selection bias

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



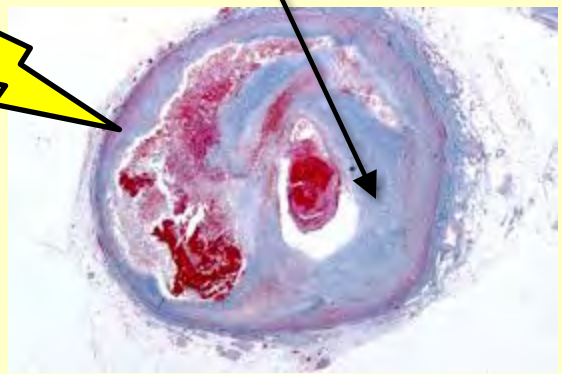
Cardiovascular Disease: Atherosclerosis





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

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

	Estimate (β)	Standard Error	*t* value	P value
(Intercept)	3.9835	0.0951	41.8834	
Age	-0.0032	0.0016	-2.0240	0.04
M	0.0788	0.0268	2.9409	0.004
E	-0.4609	0.1725	-2.6716	0.008
ahigh	0.2092	0.0989	2.1141	0.04

- 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

*oxLDL = oxidized low-density lipoprotein

DiNEH Project Results:

Discussion of Water Ingestion Findings*

- 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:

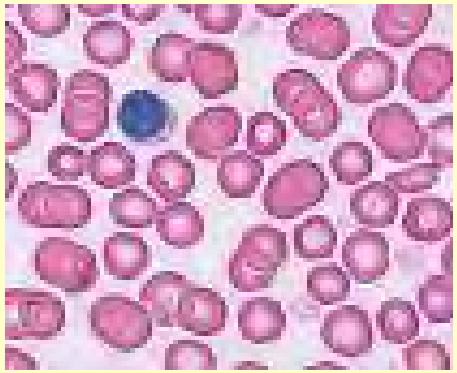
Immune system and autoimmunity

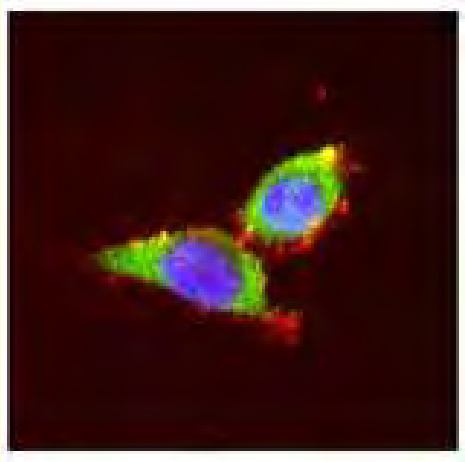
- Immune system — human body's defense system
- Processes work to ensure survival of the individual and reinstate good health conditions after infection
- Autoimmune diseases — conditions resulted in disturbance of healthy immune function, hyperactivity and overproduction of immune activation
- Studies 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

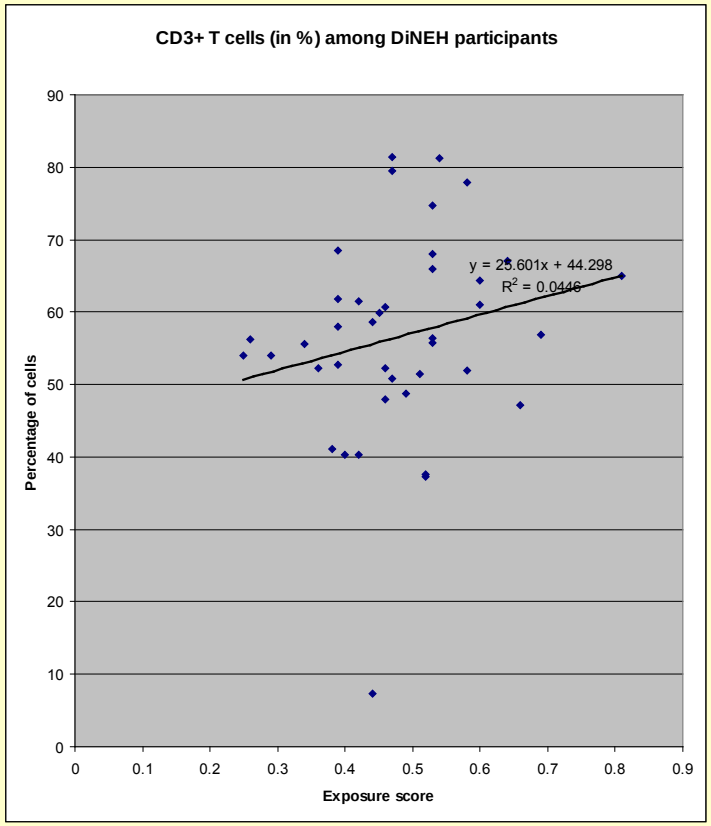
- Lymphocyte subpopulations from whole blood samples
- Becton Dickinson Simultest IMK Plus lymphocyte kit was used
- 6 cell populations were measured:
  - T cells (CD3+), T helpers (CD4+), T suppressors (CD8+);
  - B cells (CD19+)
  - HLA-DR+ cell activation in T cells
  - B cells and other cell types;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

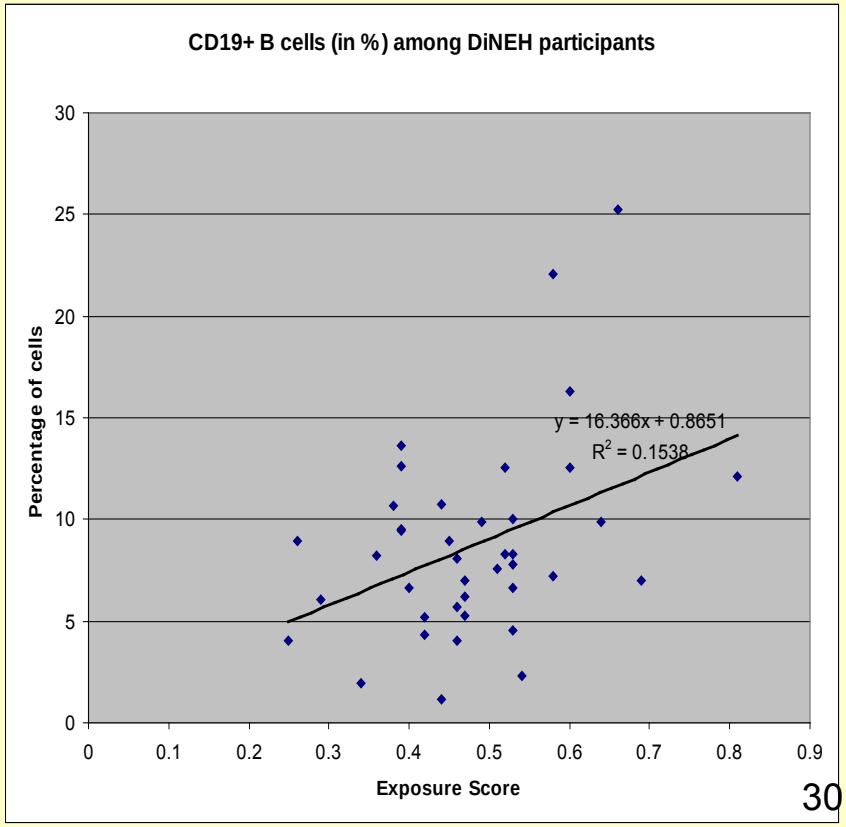




DiNEH Project flow cytometry results I

Immune impairment associated with U exposure

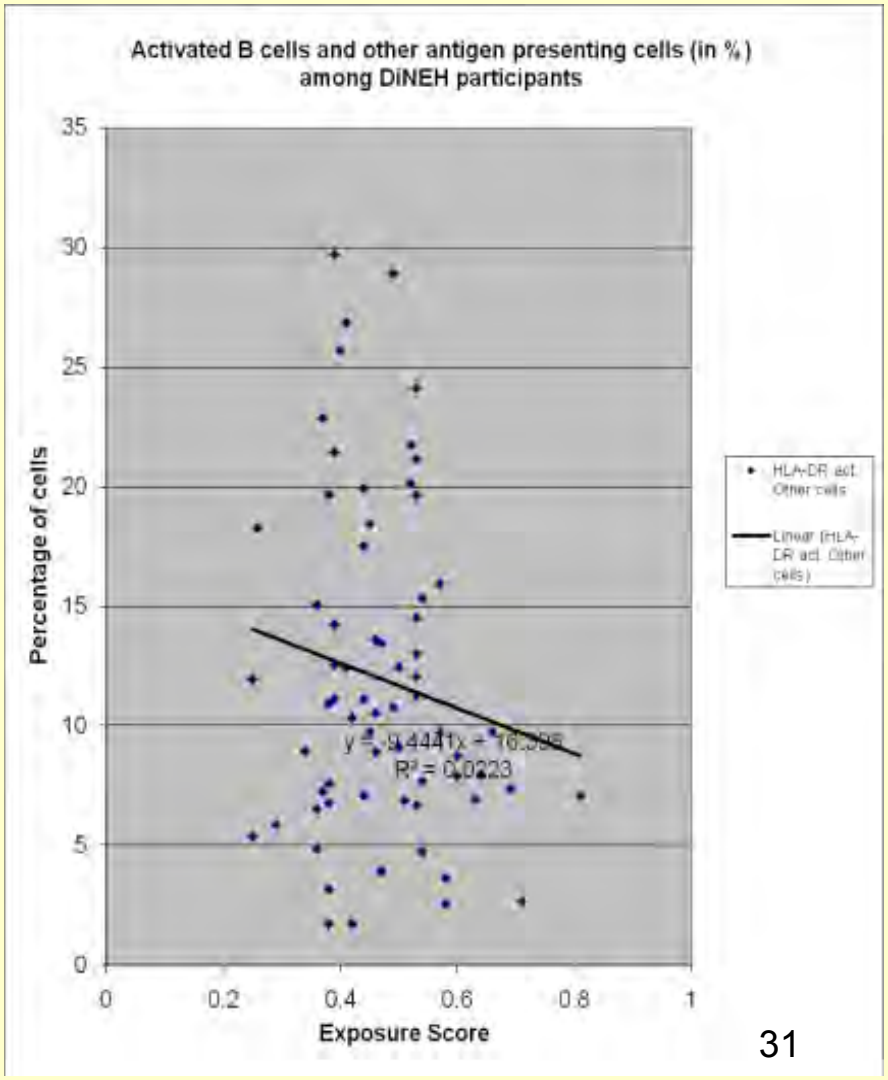




DiNEH Project flow cytometry results II

Altered immune response related with U exposure

- 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

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