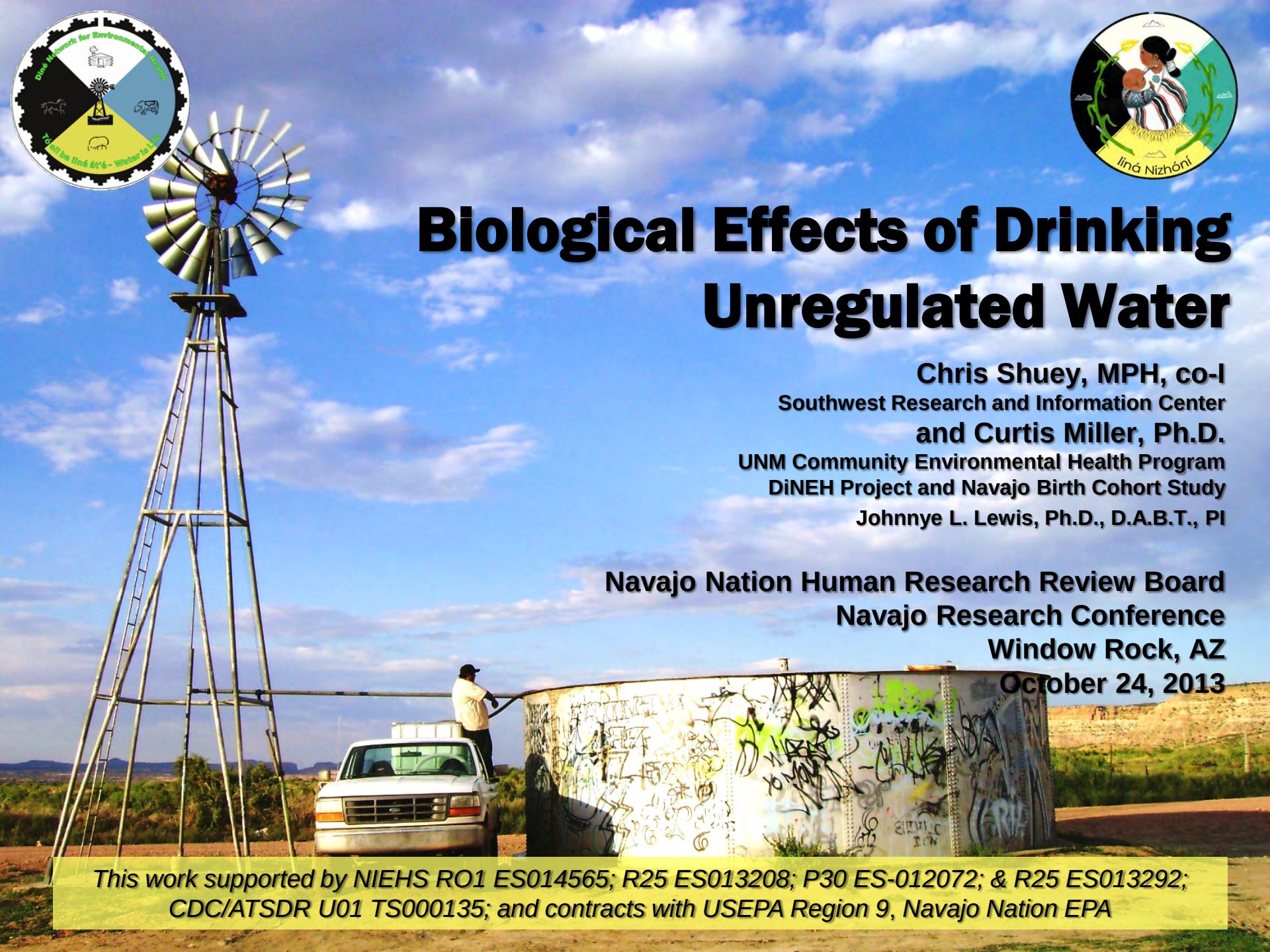

Biological Effects of Drinking Unregulated Water

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DiNEH Project and Navajo Birth Cohort Study
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Navajo Nation Human Research Review Board
Navajo Research Conference
Window Rock, AZ
October 24, 2013

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Diné Network for Environmental Health Project*

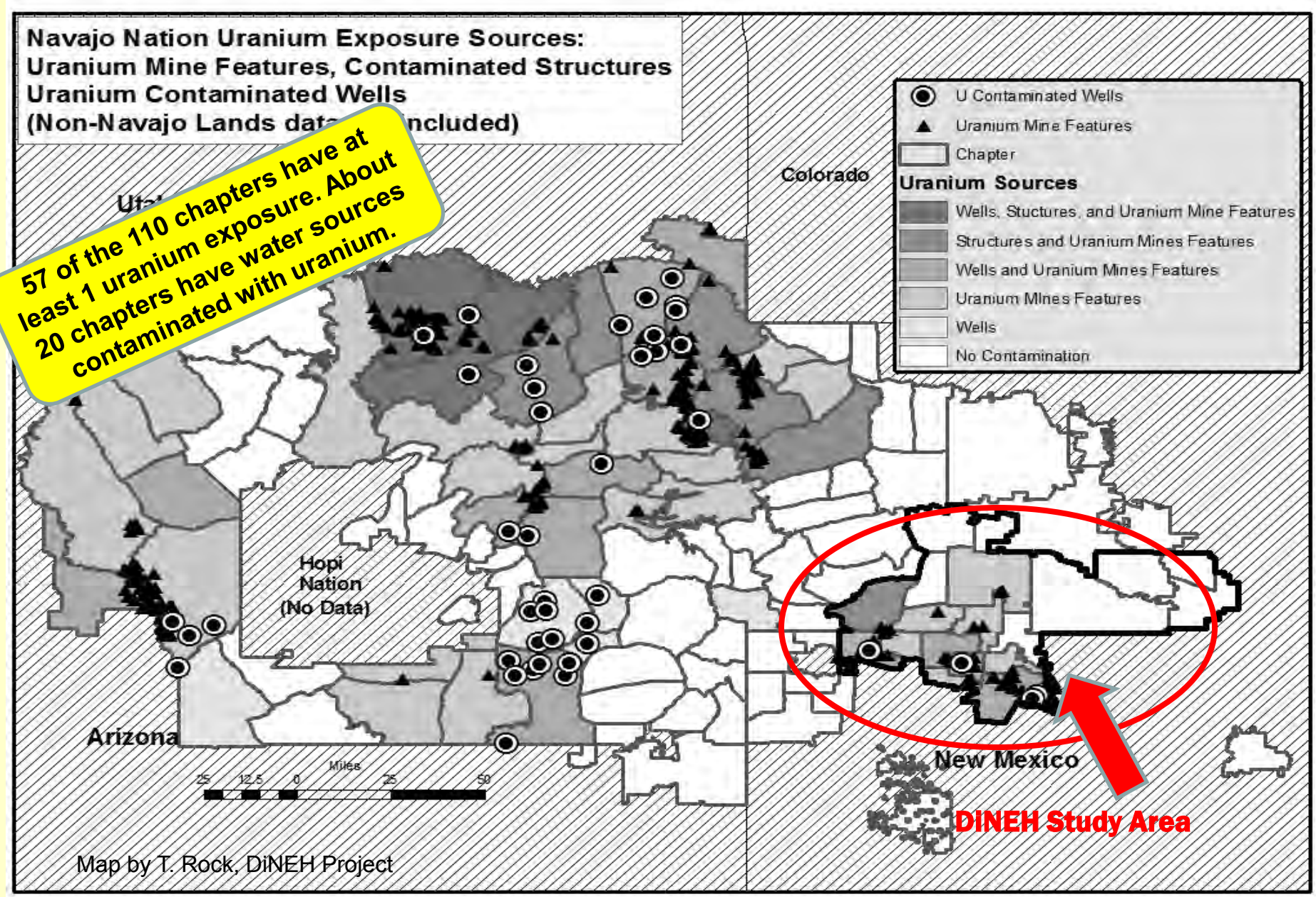
Kidney Health Project History and Purpose

(2001-2011; data analysis 2011-present)

- Original Community Question: ***Does ingesting uranium in unregulated water increase risk of kidney disease?***
  - Evolved to broadly examine environmental uranium exposures and health
- Community-based participatory research
  - Build Navajo community research capacity
  - Respect culture and language
- Commitment to inform policy and improve clinical care
  - Participation in Waxman Hearing (2007), EPA Five-Year Plan
  - Collaboration with Navajo Area IHS medical monitoring program, Community Uranium Exposure-Journey To Healing

*The DiNEH Project and Navajo Birth Cohort Study have been approved by the Navajo Nation Human Research Review Board, UNM Human Research Review Committee, Yale Univ. IRB, CDC/ASTDR IRB, and the federal OMB.

Navajo Uranium Legacy is Pervasive



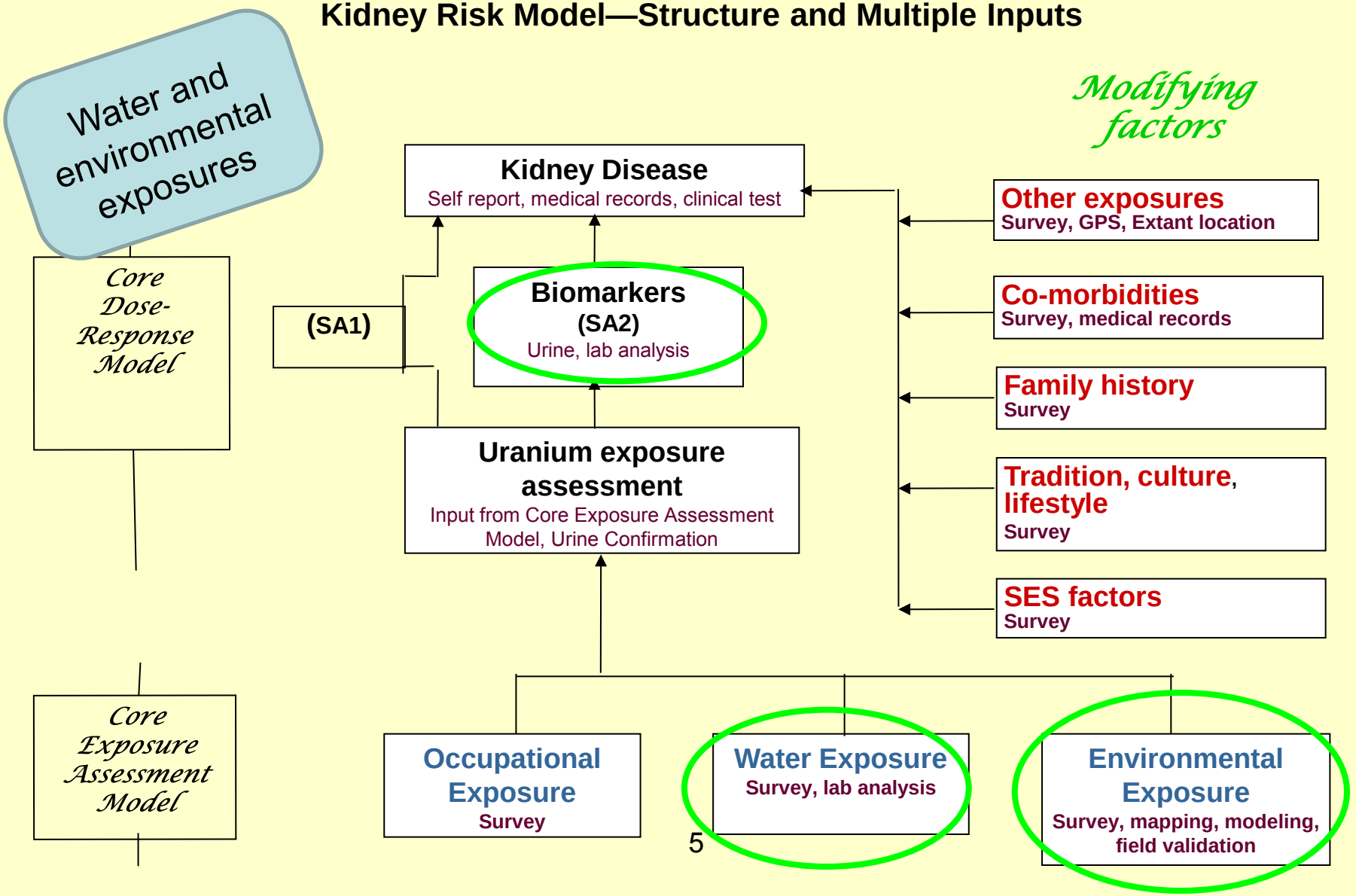
Overview of DiNEH Project Results

- 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 biological sample collection and biomarker analyses for 267 participants; laboratory and statistical analyses continuing

DiNEH Risk Model

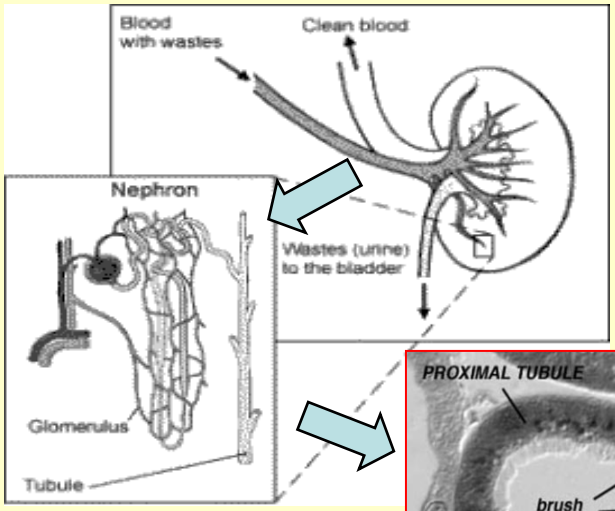
Sources of inputs to estimate participants' total exposure

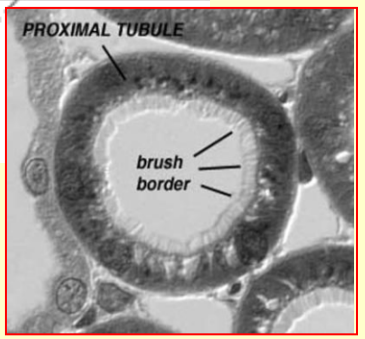
Kidney Risk Model—Structure and Multiple Inputs

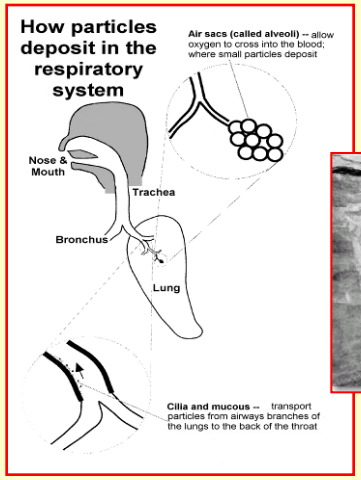


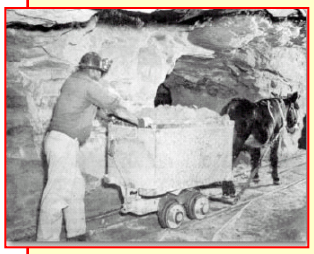
Uranium in water affects kidneys

- **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:**
  - Lung cancer from radon exposure
- Based on hundreds of studies in laboratory animals, humans and uranium workers over past 100+ years





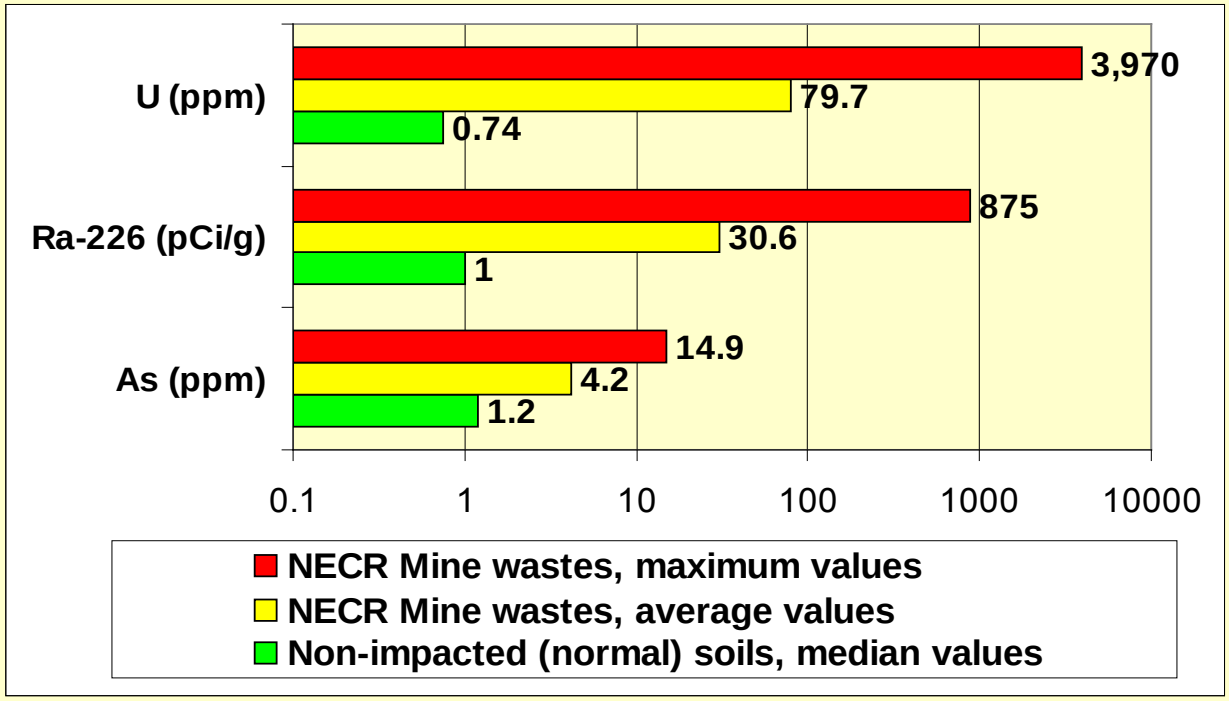




Uranium mine wastes contribute heavy metals, radionuclides to soils and may leach into groundwater


NECR Mine wastes, 2003, before partial recontoured in 2009

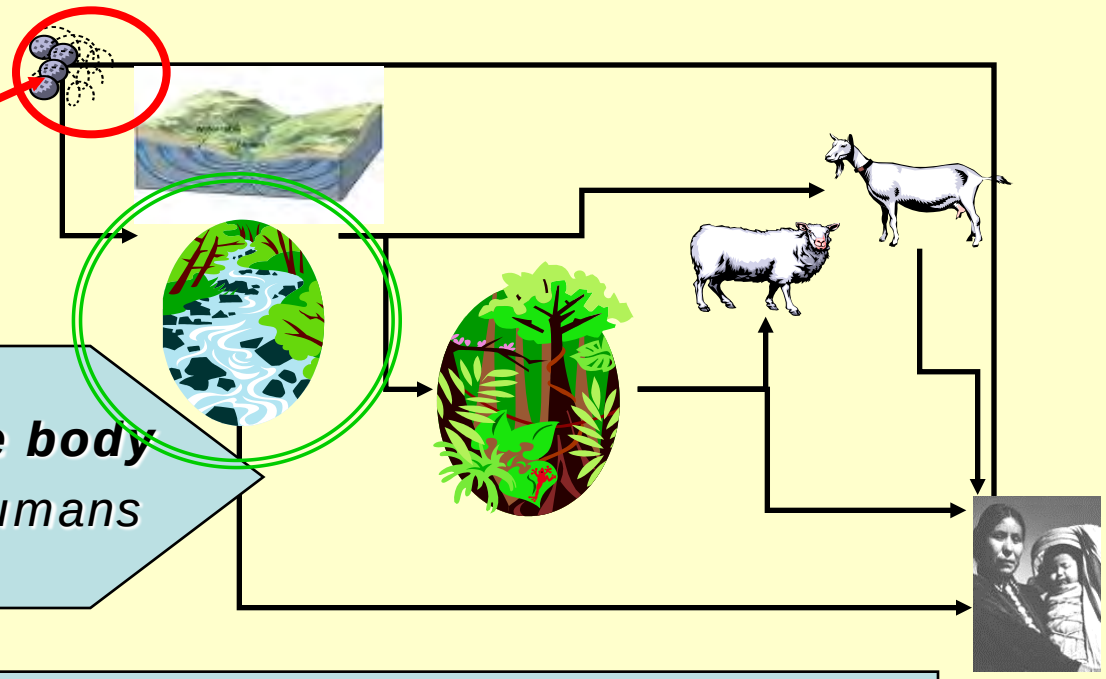

DiNEH staff collect background soil samples in Church Rock, 2005.



- Mine wastes contain (1) all the radioactive elements that decay from natural uranium, or U-238, and (2) trace metals, such as U and As, that are in the ores
- Uranium is both a heavy metal and radionuclide

Water is the pathway, ingestion the exposure route

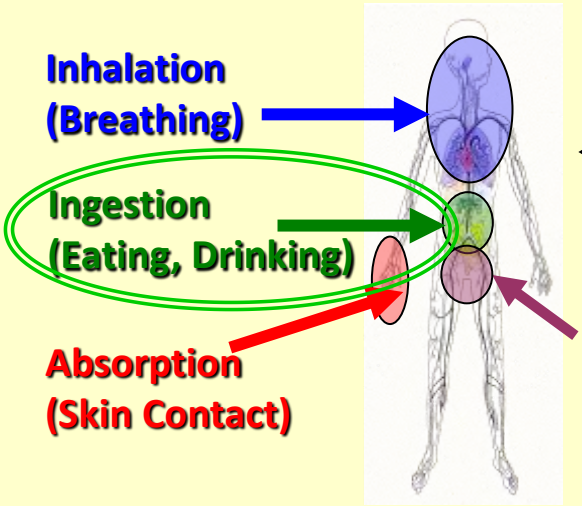
SOURCES: Potentially harmful contaminants in the environment



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

Drinking water may be significant source of exposure to uranium, other contaminants

USEPA: ~80% of uranium intake comes from drinking water

Regulated Water →

- Regularly tested and treated to ensure safety
- Complies with primary drinking water standards
- NTUA water piped to homes or available at water-hauling stations

Regulated water at two hauling stations

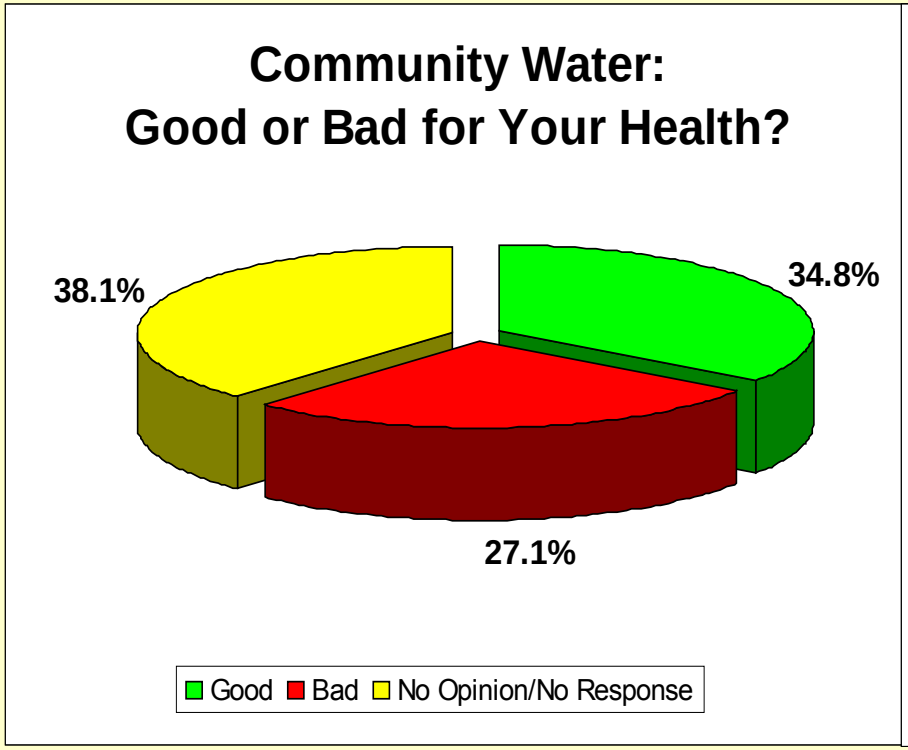
Examples of unregulated water sources

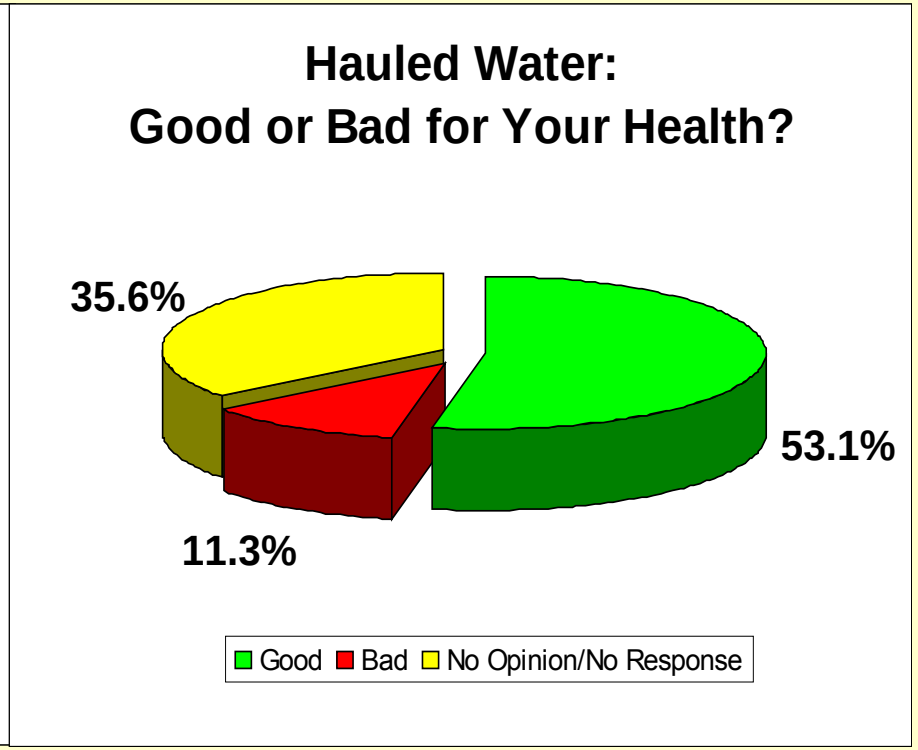
←Unregulated Water

- *Not* regularly tested or treated
- Windmills, springs, artesian wells, private wells, stock ponds, rivers
- Originally intended for livestock watering, irrigation
- Navajo Nation policy is that this water is not to be used for drinking

DiNEH Survey Results (N=1,304):

Perceptions About Water Quality





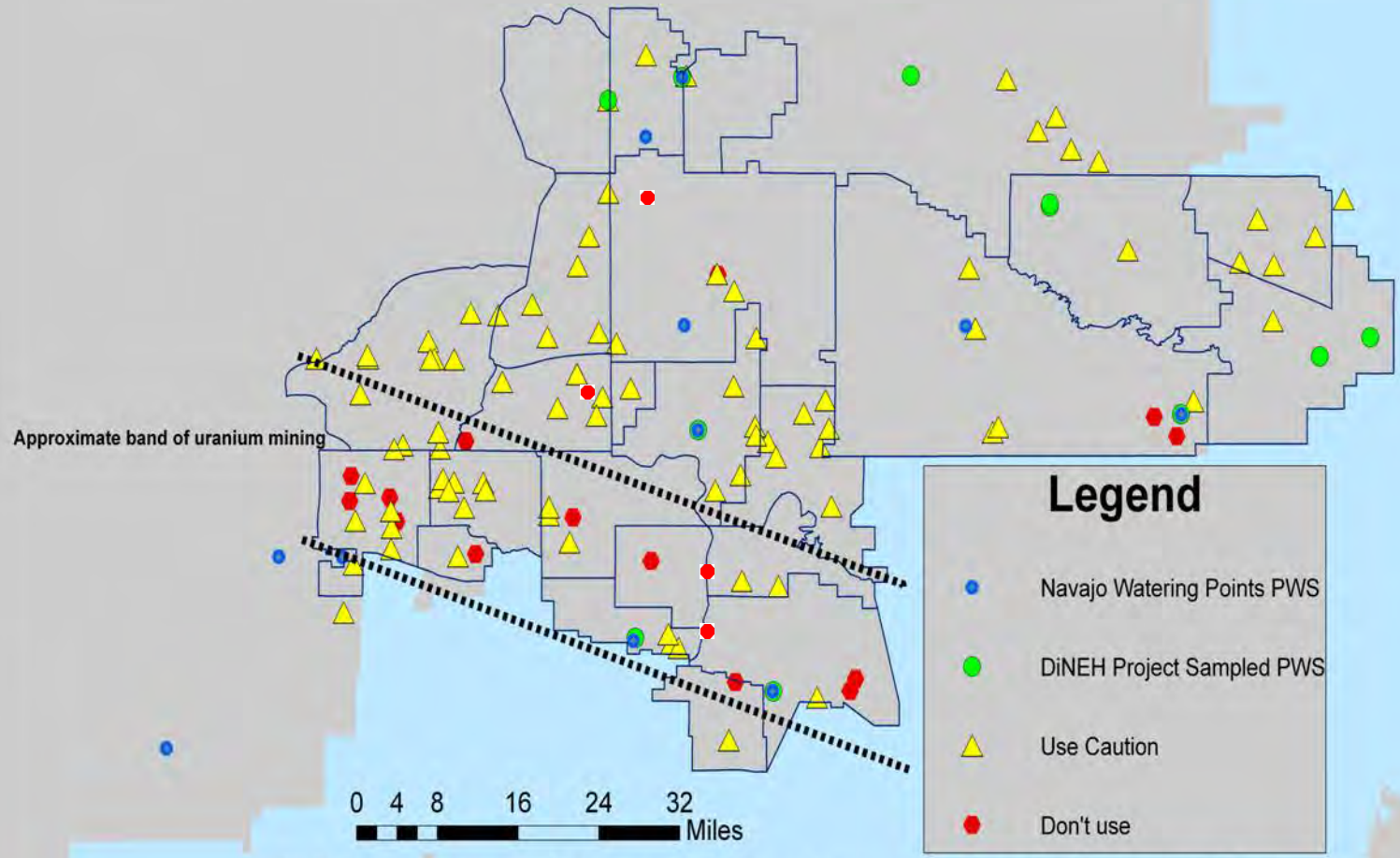
64% of DiNEH survey participants said their homes are connected to a public water system, which means 36% are NOT connected to a PWS

Water Quality Units, DiNEH Water Sampling Methods

- General chemistry, metals, solvents
  - Milligrams per liter (mg/l), or parts per million (ppm)
  - Micrograms per liter (µg/l), or parts per billion (ppb)
  - 1 mg/l = 1,000 µg/l
- Radioactivity
  - picoCuries per liter (pCi/l)
- USEPA-recommended sampling protocols
  - Field instruments, appropriate containers
  - QA/QC, chain-of-custody
  - Analyses at EPA-certified labs

Below: pH, temperature, salinity meter for field assessment; 1 liter plastic sample bottles

DiNEH Project-sampled Water Sources in the Eastern Agency: Recommendations for Human Use



DiNEH Project never recommends using unregulated water sources in the Study Area for human drinking water for two reasons:
1) We only test water for heavy metals and major ions; we do not test water for bacteria, pesticides, petroleum contaminants or solvents.
2) Navajo Nation policy is that water from "livestock-use-only" water sources is not intended for human consumption.

DiNEH Project Water Sampling Program Cumulative Results*

Water sources newly sampled (includes 11 sources connected to public water supplies)	130
Water sources sampled more than once to confirm historic or recent data	42
Water sources exceeding 1 or more Maximum Contaminant Levels (MCLs) on at least one occasion	28
Most common toxic contaminants detected (at or above their respective MCLs)	As (10) U (9)
Water sources exceeding 1 or more SDWSs	107

*Includes water sources sampled by Church Rock Uranium Monitoring Project, 2003-2007, and USEPA 2008-2010.

DiNEH water quality statistics compared with USEPA data

(Concentrations in mg/l; Radium and Gross Alpha in pCi/l)

DiNEH* (2003-2010)

	MCL	N	Range	Mean ± SE	Med.
As	0.01	158	0-0.482	0.007±0.0399	0.00094
U	0.03	135	0-0.26	0.009±0.0286	0.00027
Se	0.05	157	0-1.0	0.014±0.082	0.0011
F	4.0	192	0-8.3	1.15±1.177	0.705
Ra	5.0	84	0-10.9	1.87±2.10	1.28
NO_2	1.0	118	0-7.5	0.131±0.694	0
NO_3	10.0	199	0-29.0	1.583±3.54	0.3
GA	15	26	0.3-21.7	5.15±5.012	3.99
Pb	.015	154	0-0.021	.0012±0.0028	0

*All water sources in 20 chapters of Eastern Agency
GA = Gross alpha radioactivity

USACE-USEPA* (1994-2000)

	MCL	N	Range	Mean ± SE	Med.
As	0.01	222	0-0.282	0.009±0.028	0.0028
U	0.03	222	0-0.286	0.0198±0.039	0.0076
Se	0.05	222	0-0.957	0.0094±0.07	0
Ra	5.0	222	0-8.2	0.56±1.7	0.6
GA	15.0	222	0-155	11.5±21.98	4.4
Pb	0.015	222	0.0.1	0.0029±0.012	0
Al**	0.05-2.0	222	0.61.9	0.84±4.8	0.08

*All water sources located in the western half of the Navajo Nation in Arizona and Utah
**Aluminum has no MCL; the reference range given is the secondary drinking water standard
GA = Gross alpha radioactivity

Major water contaminants found in DiNEH Project water sources and their principal health effects

(number of sources exceeding MCLs shown in parentheses)

Arsenic (10)	Skin pigment effects; increased risks of skin, lung, bladder cancers; interaction with UV; nerve damage; nervous system, kidney, cardiovascular system effects
Uranium (9)	Kidney toxicity; increased cancer risks as alpha-emitting radionuclide
Selenium (7)	Bone, fingernail loss; circulation effects (numbness in fingers and toes); irritability, GI distress; essential nutrient at low doses
Fluoride (6)	At high doses can cause mottling of teeth, disturb bone formation — low doses added for dental health!
Radium (5)	Bone cancer; teeth decay
Nitrite (4)	Blue-baby syndrome — blocks delivery of oxygen to tissue
Nitrate (3)	Blue-baby syndrome — blocks delivery of oxygen to tissue
Gross alpha (3)	Increased risk for cancer — organ systems affected depend on specific radionuclides
Mercury (2)	Kidney damage
Lead (1)	Kidney problems, high blood pressure in adults; learning disabilities in children

Blood and urine collections among DiNEH Participants by Chapter

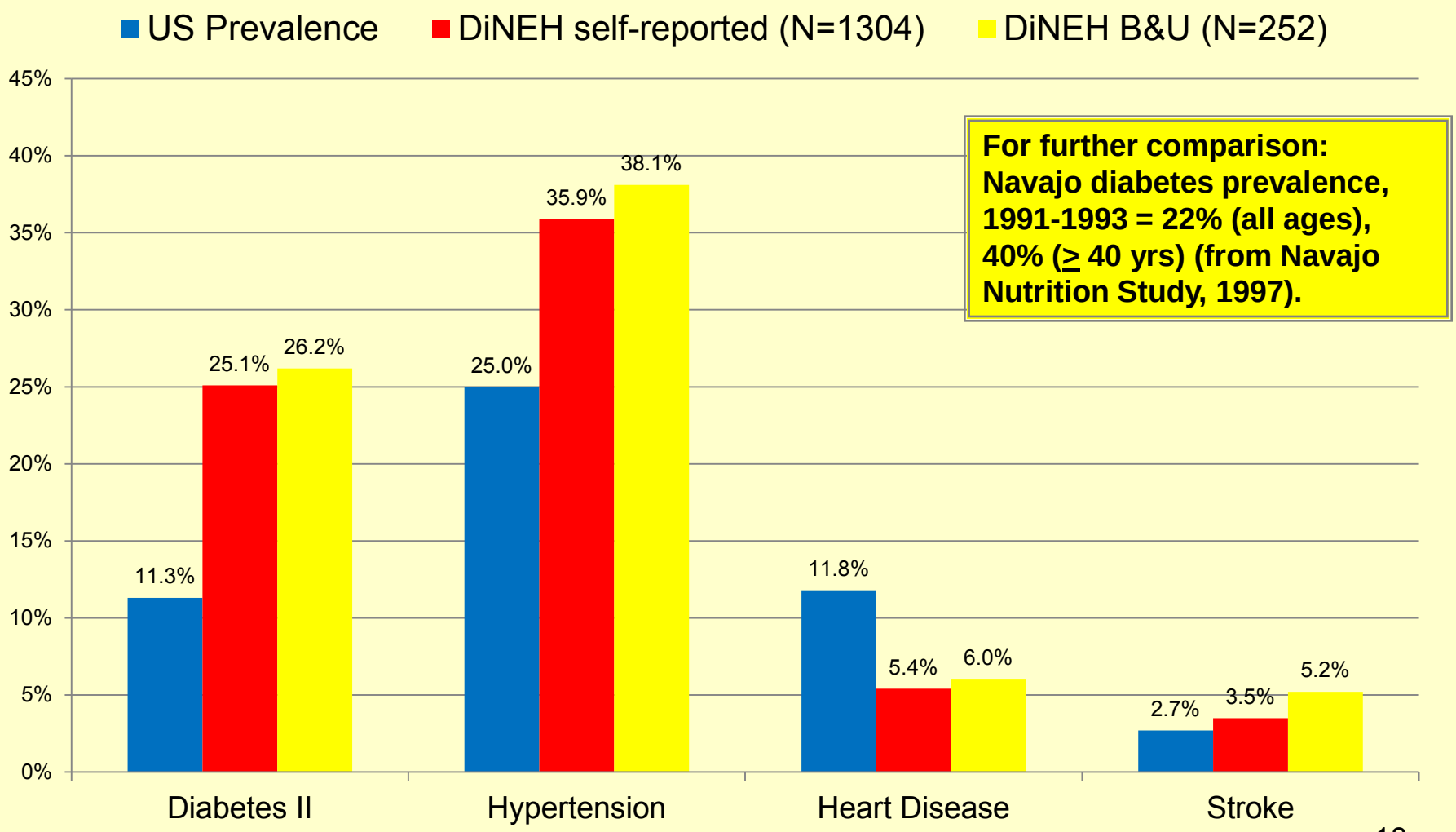
Chapter	# DiNEH Survey Participants (1,304)	# DiNEH Participants in B&U Collections (267)
Baca-Prewitt	96	32
Becenti	60	22
Casamero Lake	70	14
Church Rock	69	13
Coyote Canyon	65	18
Crownpoint	71	20
Iyanbito	61	17
Lake Valley	61	9
Littlewater	65	11
Mariano Lake	69	19
Nahodishgish	60	15
Ojo Encino	65	2
Pinedale	64	5
Pueblo Pintado	65	9
Smith Lake	69	19
Standing Rock	72	17
Thoreau	66	18
Torreon	67	0
White Rock	26	1
Whitehorse Lake	63	6

Uranium screening clinic conducted at Iyanbito Chapter in cooperation with NAIHS Community Uranium Exposure-Journey To Healing Program, April 5, 2011

Why collect blood & urine samples?

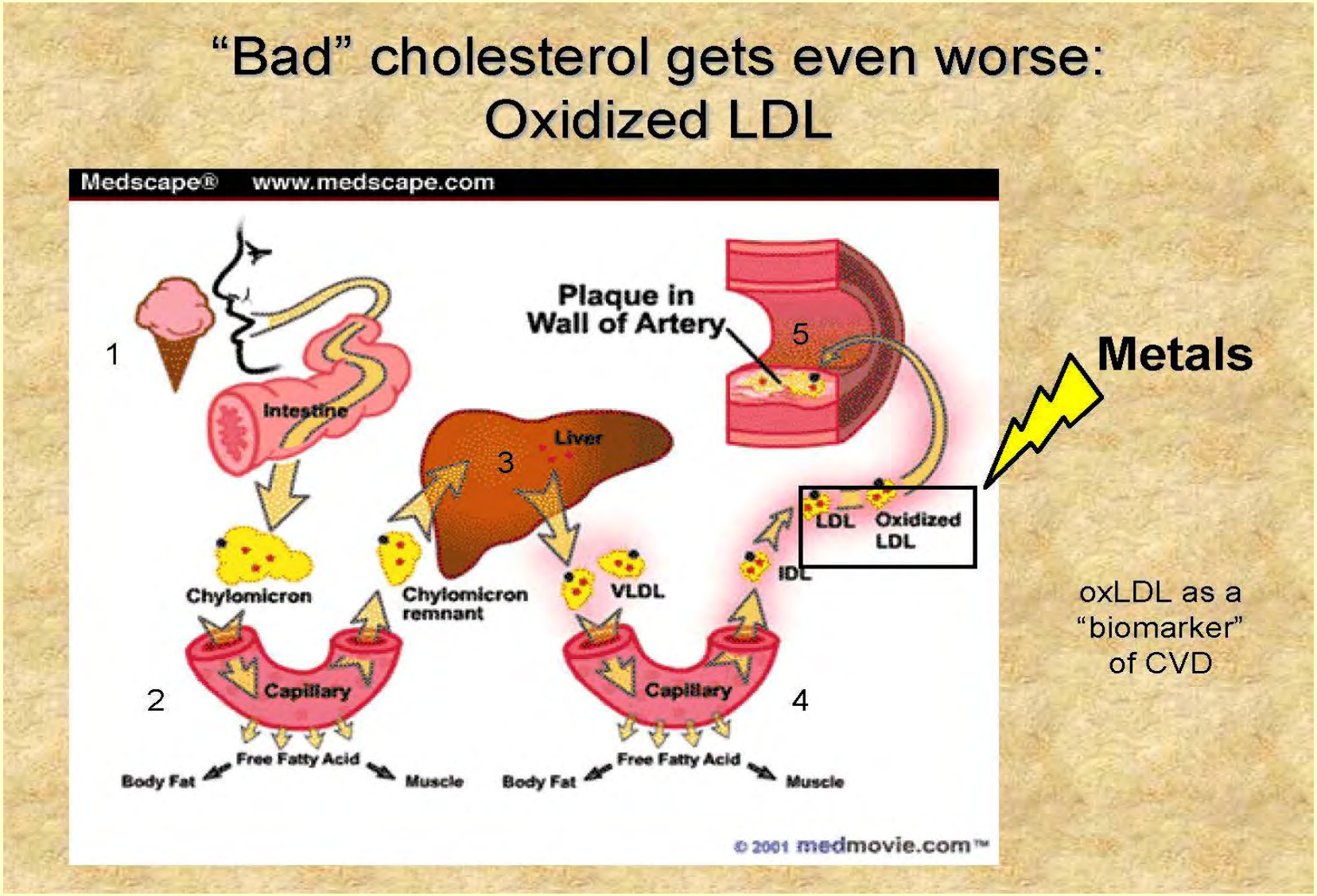
- Obtain biological confirmation of self-reported health conditions (from survey responses)
- Assess overall health of study participants by testing for clinical measures of disease
- Assess blood and urine for biomarkers of early disease
  - Biomarkers – any biological indicator of a particular disease state or stage of disease
- Investigate disease status in relation to environmental exposures
  - Hypertension (i.e., high-blood pressure)
  - **Cardiovascular disease (CVD) (i.e., atherosclerosis)**
  - Kidney disease
  - Autoimmune disease
- Recent DiNEH findings:
  - Proximity to AUMs and lifetime contacts with uranium wastes increase risk of hypertension and autoimmune disease during the environmental legacy period and kidney disease during the active-mining era; See: http://www.epa.gov/region09/superfund/navajo-nation/pdf/stakeholders/2013/dr-lewis-di-neh-project-update2013.pdf

Disease prevalences among DiNEH participants compared with US Rates



For further comparison: Navajo diabetes prevalence, 1991-1993 = 22% (all ages), 40% (≥ 40 yrs) (from Navajo Nutrition Study, 1997).

Metal exposures (from water or wastes) may contribute to cardiovascular disease (CVD)

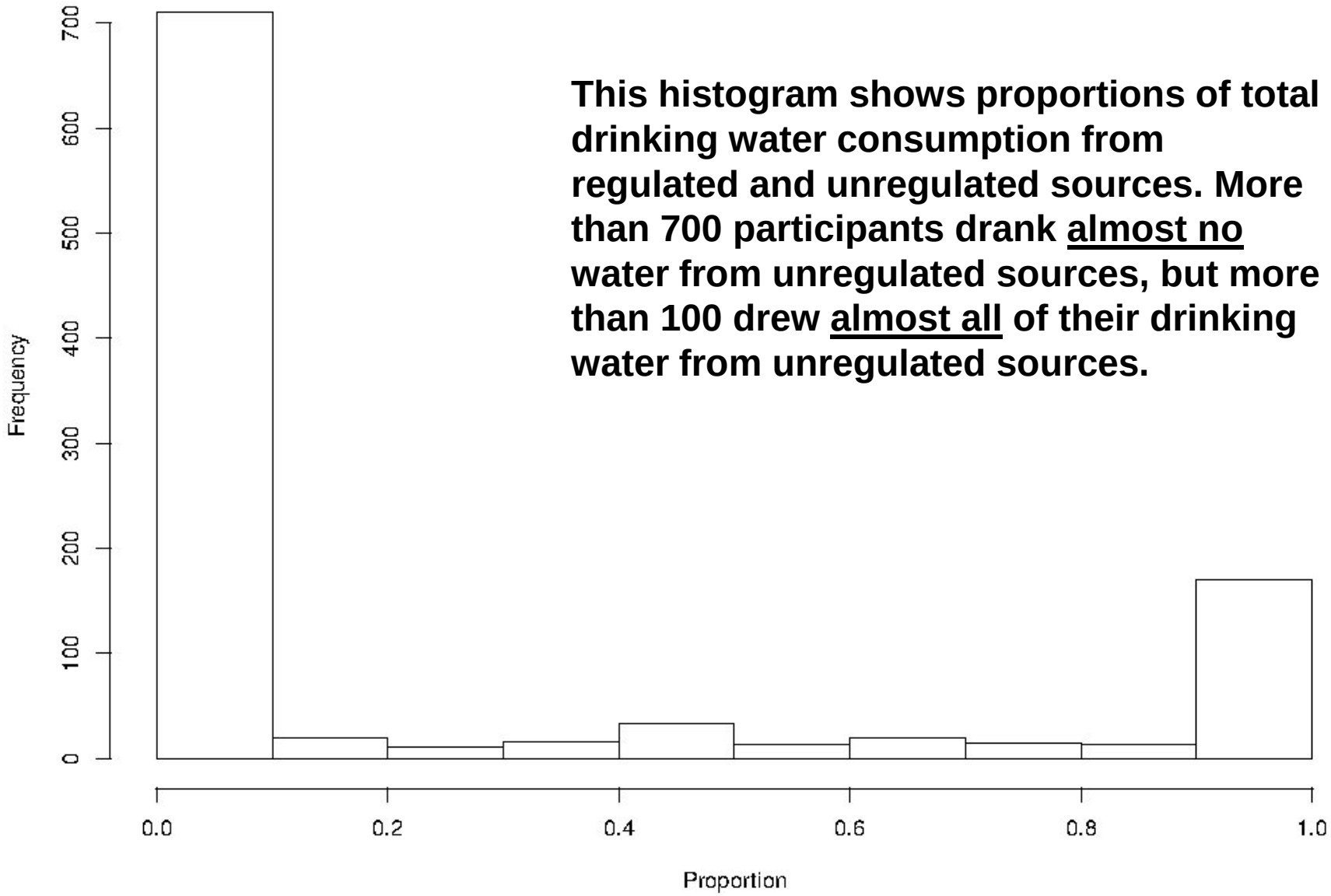


Statistical analyses of DiNEH water quality data and cardiovascular biomarker results*

Curtis Miller, Ph.D.

*Biomarker results were contributed by Molly Harmon and Matthew Campen, Ph.D., UNM College of Pharmacy, who carried out the laboratory analyses of CVD markers discussed in this section. Their work is referenced at the end of this presentation.

Proportion of drinking water coming from unregulated sources among 1,304 participants



This histogram shows proportions of total drinking water consumption from regulated and unregulated sources. More than 700 participants drank almost no water from unregulated sources, but more than 100 drew almost all of their drinking water from unregulated sources.

How we calculated a participant's annual intake of arsenic (As) and uranium (U) from survey responses

If participant *j* listed `Well X' as a source, determine

- $N_{X,j}$ = number of trips to Well X in a year by *j*
- $Q_{X,j}$ = Mean quantity of water drawn in a trip to Well X by *j*
- $P_{X,j}$ = proportion of water from Well X used by *j* for drinking and cooking
- U_X (As_X)= Concentration of uranium (arsenic) in Well X

→ Then, annual intakes of metals by *j* from Well X are

$$UI_{X,j} = N_{X,j} * Q_{X,j} * P_{X,j} * U_X$$

$$AI_{X,j} = N_{X,j} * Q_{X,j} * P_{X,j} * As_X$$

→ And the annual intakes of metals by participant *j* are

U_j = Sum of $UI_{X,j}$ over all wells X from which *j* draws water

As_j = Sum of $AI_{X,j}$ over all wells X from which *j* draws water

Hypothetical example of calculation of one participant's annual intake of U

1) Participant drew water from wells 4266, 4044, and 2507.

2) 4266 and 4044 were visited once a month, 2507 once a week.

3) The average amount of water drawn at each visit was six liters for well 4266, 20 liters for well 4044, and 10 liters for well 2507.

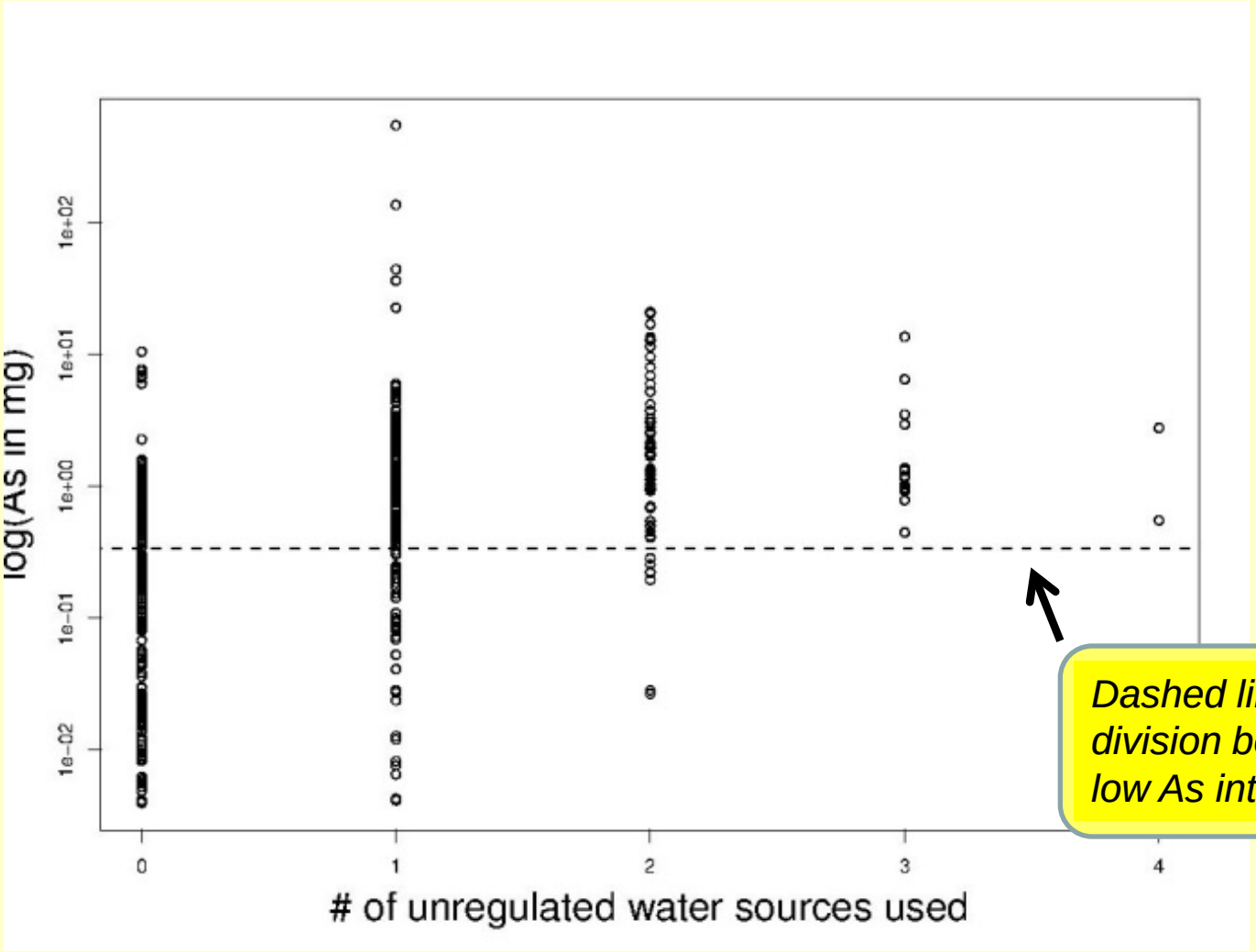
4) The proportion used for drinking or cooking: 50% for well 4266, 80% for well 4044, and 10% for well 2507.

5) The concentrations of uranium (milligrams per liter (mg/l)): 0.054 for 4266, 0.025 for 4044, and 0.0005 for 2507.

6) Estimated annual uranium intake of participant was then:

(12*6*0.5*0.054) + (12*20*0.8*0.025) + (52*10*0.1*0.005) = **6.77 milligrams (mg)**

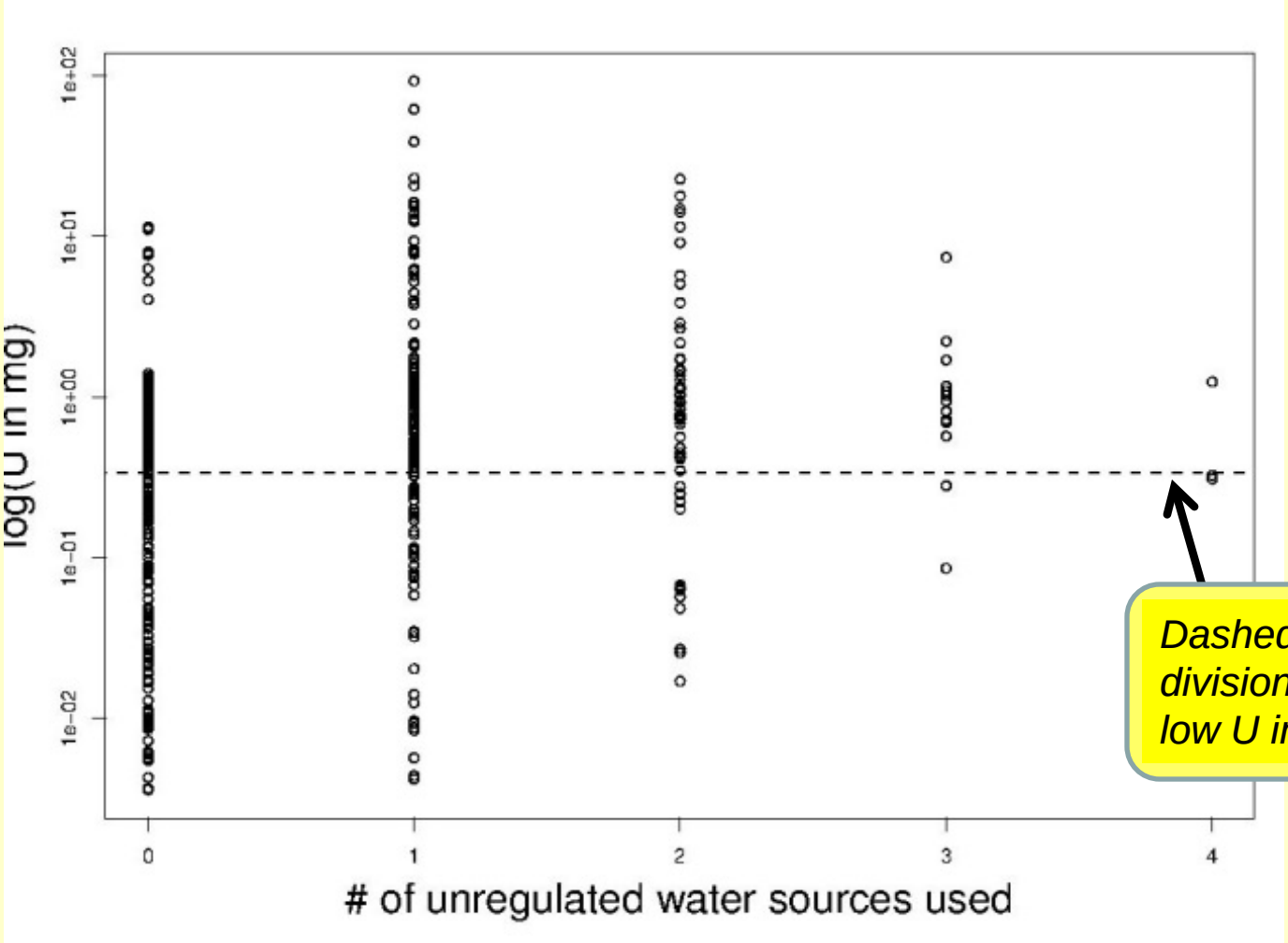
Unregulated water sources contributed most of annual intake of arsenic



Graph shows log of estimated annual arsenic intake plotted against number of unregulated sources used by participants. Participants who did not use unregulated water sources, overall, had lower annual As intake.

Dashed line indicates division between high and low As intake (1.4 mg)

Unregulated water sources contributed most of annual intake of uranium

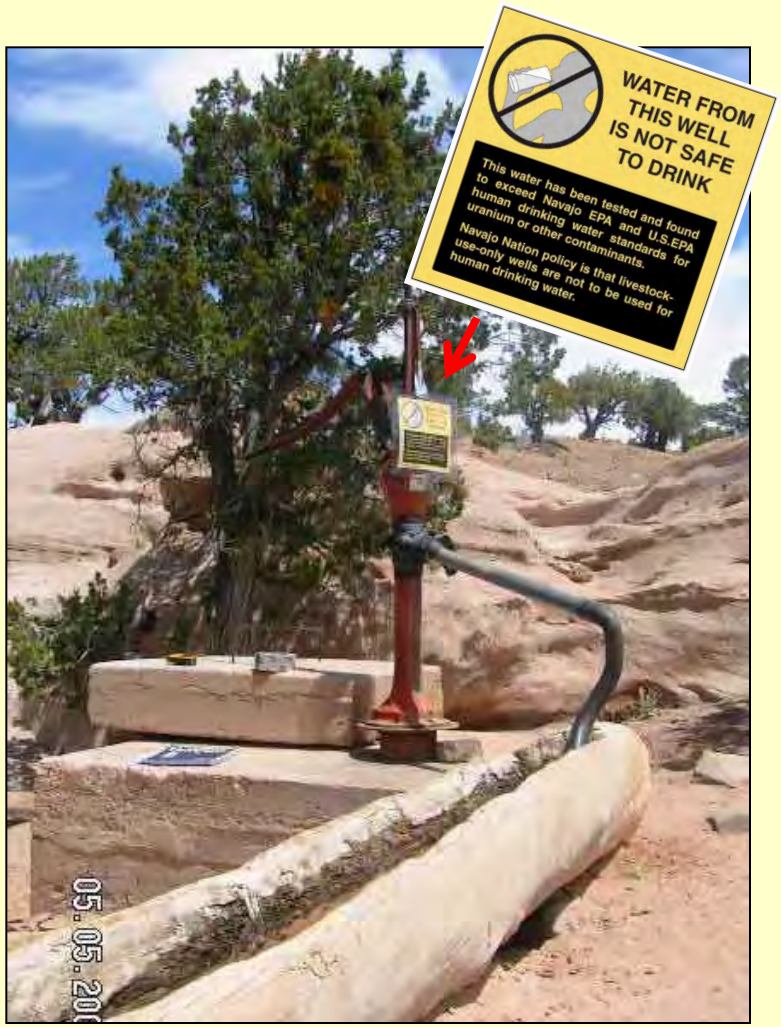


Graph shows log of estimated annual uranium intake plotted against number of unregulated sources used by participants. Participants who did not use unregulated water sources, overall, had lower annual U intake.

Dashed line indicates division between high and low U intake (1.4 mg)

Unregulated water sources contributed high annual intakes of As and U

- For both metals, high annual intakes occurred *only* for participants who drew water from at least one unregulated source
- Maximum annual intakes were calculated as ~550 mg for As, ~92 mg for U
- "High" annual intake defined as ≥1.4 mg of either As or U



This developed spring in Church Rock had the highest uranium level, 0.26 mg/l, of any water source in the study area, or nearly 10 times greater than the MCL of 0.03 mg/l. A warning sign was posted in 2008.

Blood and urine sample collection and statistical analysis methods

Sample Collection

- Urine and blood samples collected from 267 participants in the DiNEH survey
- Samples tested for clinical parameters by Northern Navajo Medical Center staff and for assayed for research biomarkers by UNM staff
- Biomarker laboratory analyses done at UNM, University of Texas-Houston

Statistical Analyses

- Logistic regression and Bayesian analyses used to model more than 40 biomarkers
- *Model selection* used to derive a reduced regression model for biomarkers of interest

Findings of Statistical Analyses

	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

- Age, occupational exposures (M), distance to and contacts with mine wastes (E), and annual arsenic intake from drinking water were *significant predictors* of oxLDL
- High annual arsenic intake was significantly associated with the log of oxLDL (*p=0.0054)*
  - This is the probability that *ahigh* was not associated with oxLDL, and the apparent effect of *ahigh* had occurred by chance
- Small p-value of *ahigh* for oxLDL indicates the association of *ahigh* to oxLDL is real

Discussion of Key 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

*Sources: 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.

Discussion and Conclusions

Chris Shuey, MPH, co-investigator

What do we know about unregulated water sources on the Navajo Nation?

Background: Navajo Nation

- Beverly Becenti-Pigman, NNHRRB chair, appointed *ad hoc* task force in 2010 to collaborate and compile water quality data
- Navajo Department of Water Resources (NDWR) identified 2,274 water sources: 889 windmills + 1,393 dug wells and developed springs (C. Mike, April 2013)
- Some water quality data exist in NDWR files, but not systematically compiled
- NDWR scanning records to add to Wells Database

Collaborations and Actions

- SRIC, Diné Environmental Institute, NNEPA, NDWR, USEPA met to discuss sources of water quality data
- At least 20 different sources of data identified
  - Published reports
  - Agency files
  - Special sampling
- SRIC began compiling data in 2011; work continued into 2013
  - Water quality data identified for more than 550 different water sources
- No dedicated funding

Arsenic, uranium most frequent contaminants in Navajo Nation water sources

Total number *different* 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%

*MCL = maximum contaminant level, EPA standard for safe drinking water

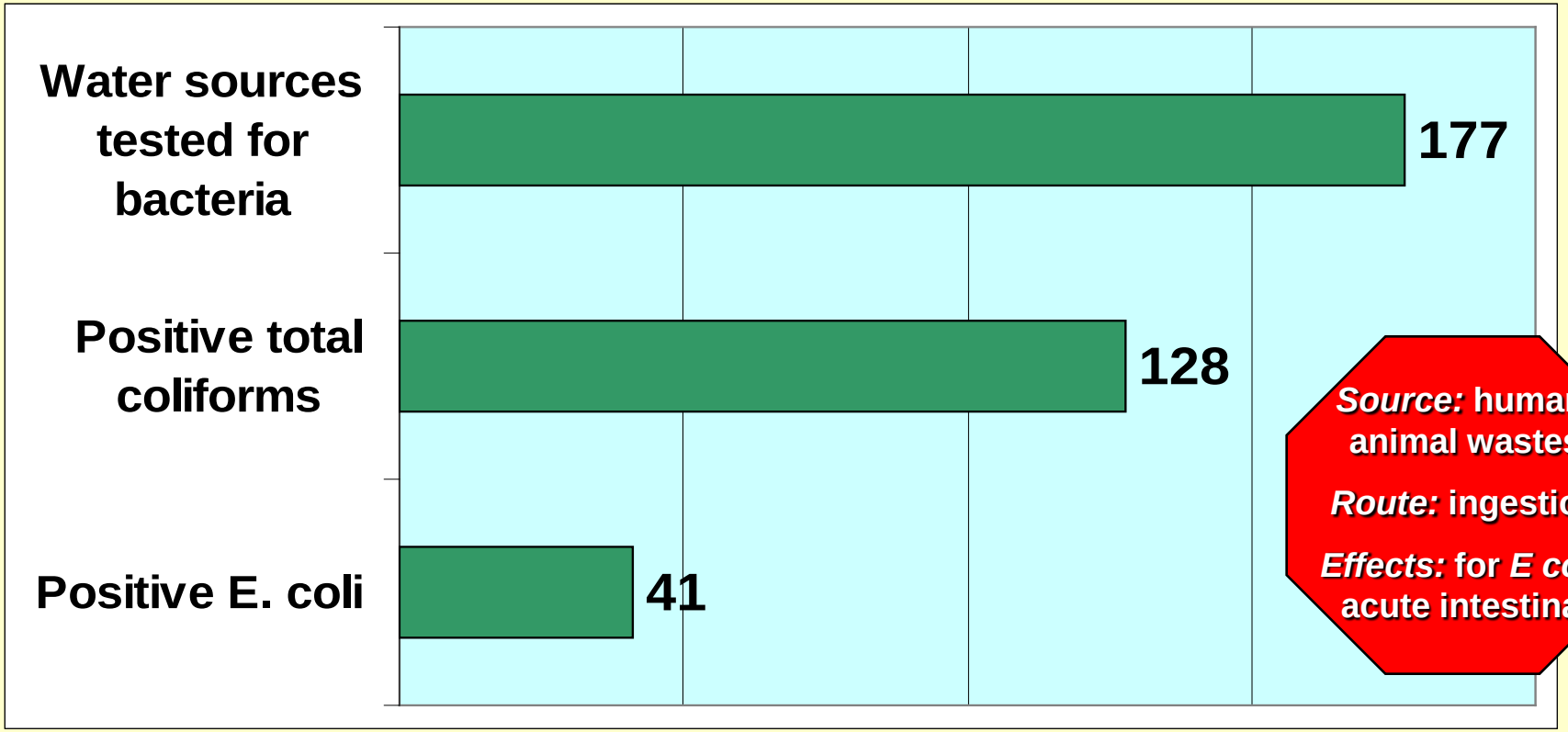
Data in table to left includes results through January 2012

Data Sources:

- DiNEH Project + CRUMP sampling, 2003-2010; 130 sources in ENA
- CDC-NDOH sampling, 2006-2007; 211 sources in central Navajo (AZ)
- USACE-USEPA sampling, 1994-2000; 222 sources in Arizona
- Diné Environmental Institute sampling, 2008-2010; 66 sources in Four Corners Area
- USEPA re-sampling, 2008-2010; 100 sources

Unregulated water sources have high occurrence of harmful bacteria

Tests Done by CDC and NDOH in Central Navajo, 2006-2007



*Based on presence of total coliforms; more harmful *E. coli* were present in about 23% of water sources. More information: http://water.epa.gov/drink/contaminants/basicinformation/ecoli.cfm.

NAVAJO NATION POLICY: Unregulated water sources are not to be used for human drinking. NO TESTING YET FOR PETROLEUM PRODUCTS OR PESTICIDES.

Regulated Watering Points on the Navajo Nation

Legend

- Regulated Watering Points
- Navajo Chapters
- Highway

Large areas of the Navajo Nation have NO clean-water hauling stations

Call Navajo EPA 928-871-7755 for a list of safe drinking water hauling points near you.

Miles
0 10 20 40 60 80

Communicating water risk to avoid health risks

DiNEH Project commissioned by USEPA-9 in 2008 to generate maps showing water sources contaminated with uranium and radionuclides, per congressional directives.

Navajo Birth Cohort Study will test unregulated water sources

- NBCS enrolling pregnant women to investigate possible effects of uranium exposures on pregnancies and child development
- NBCS enrollment survey: *What water sources do you drink from?*
- If participant uses unregulated water sources,
  1) access existing water quality data;
  2) if no existing data, **sample water source used by participant**
- Conduct field tests for temperature, pH, conductivity
- Collect, ship samples to USEPA-9 lab; observe QA/QC
- Analyze for:
  - General chemistry, heavy metals
  - Radionuclide analyses not funded
- Report results to participants
  - Recommend NO USE if any MCL is exceeded
- Make referrals to NNEPA

Conclusions

- Human use of unregulated water sources for drinking water continues despite water-line extensions, water-hauling programs
- Arsenic and uranium are two most frequent contaminants in unregulated water sources
- Unregulated water sources contributed vast majority of As and U intake among DiNEH participants
- Arsenic intake strongly associated with a biomarker of inflammation in the cardiovascular system, oxLDL, among DiNEH participants
- Uranium intake weakly associated with CVD marker; further biological investigations needed
- Navajo Birth Cohort Study will test unregulated water sources if pregnant women identify them as drinking water sources and no water quality data exist

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