Additions to the Medical Geologist's Toolbox

Application of Sensitive Biological Endpoints to Assess Human Exposure and Potential Health Effects at Low Environmental Contaminant Concentrations

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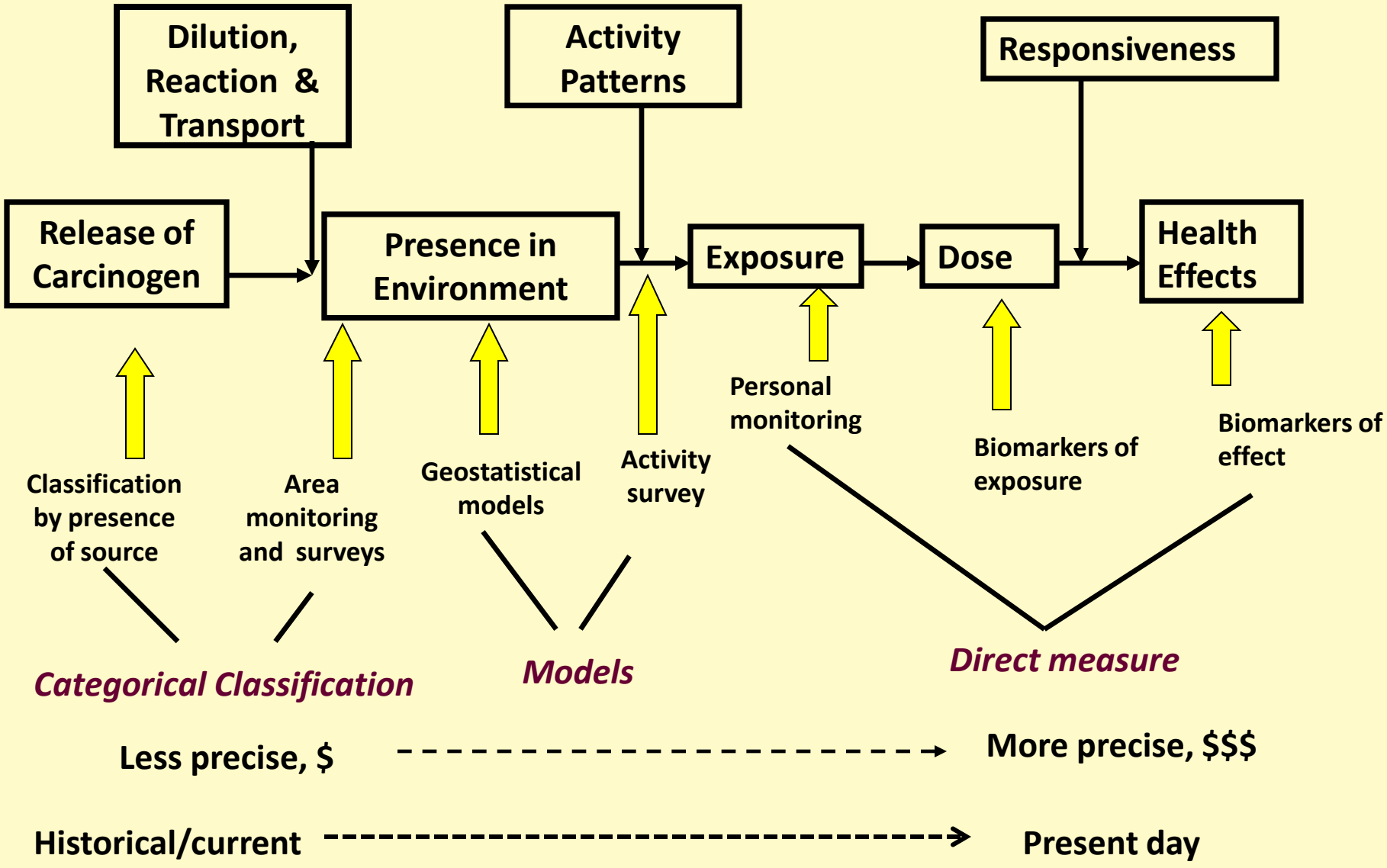
Geological Society of America 2018 Annual Conference
November 5, 2018

Outline

- Exposure assessment methods and limitations for environmental contaminants
- Description of UNM project to study potential synergistic effects of arsenic and UV exposure in promoting skin cancer
  - Case-control design
  - Exposure assessment
    - Direct measures of exposure to arsenic
    - Use of databases for estimation of historical arsenic and UV exposures
    - Internal exposure biomarkers
  - Biological endpoints for health effects
    - Comet assay
    - Single Nucleotide Polymorphisms
- Conclusions and acknowledgements

Methods for Exposure Assessment



UNM Pilot Study: As + UV -> melanoma

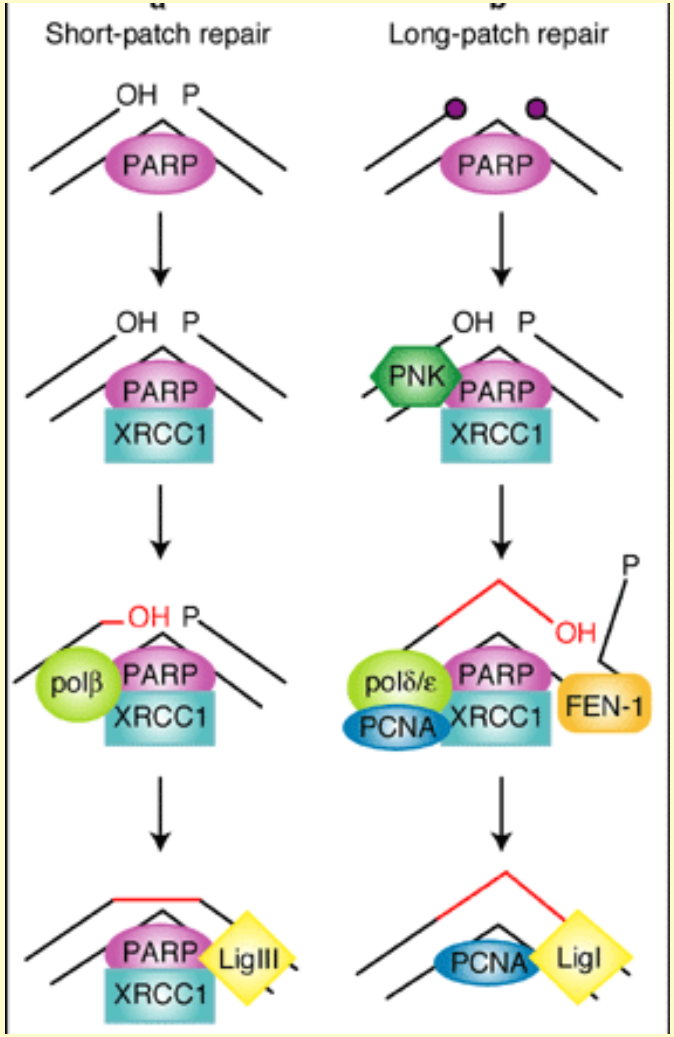
- Melanoma
  - US. 60,00 cases; 8,000 deaths annually
  - 20 – 30 yr latency
  - NM has relatively high UV due to high elevation and low latitude
  - Oxidant formation and UV-induced DNA damage
  - NM ranked 3rd in US (2002-06) in melanoma incidence in non-Hispanic whites

- Arsenic
  - NM historical iAs levels in drinking water 50-240 µg/L
  - Induces ROS DNA damage
  - Shown to be co-carcinogenic with UV in induction of non-melanoma skin cancer in epi studies & animal models

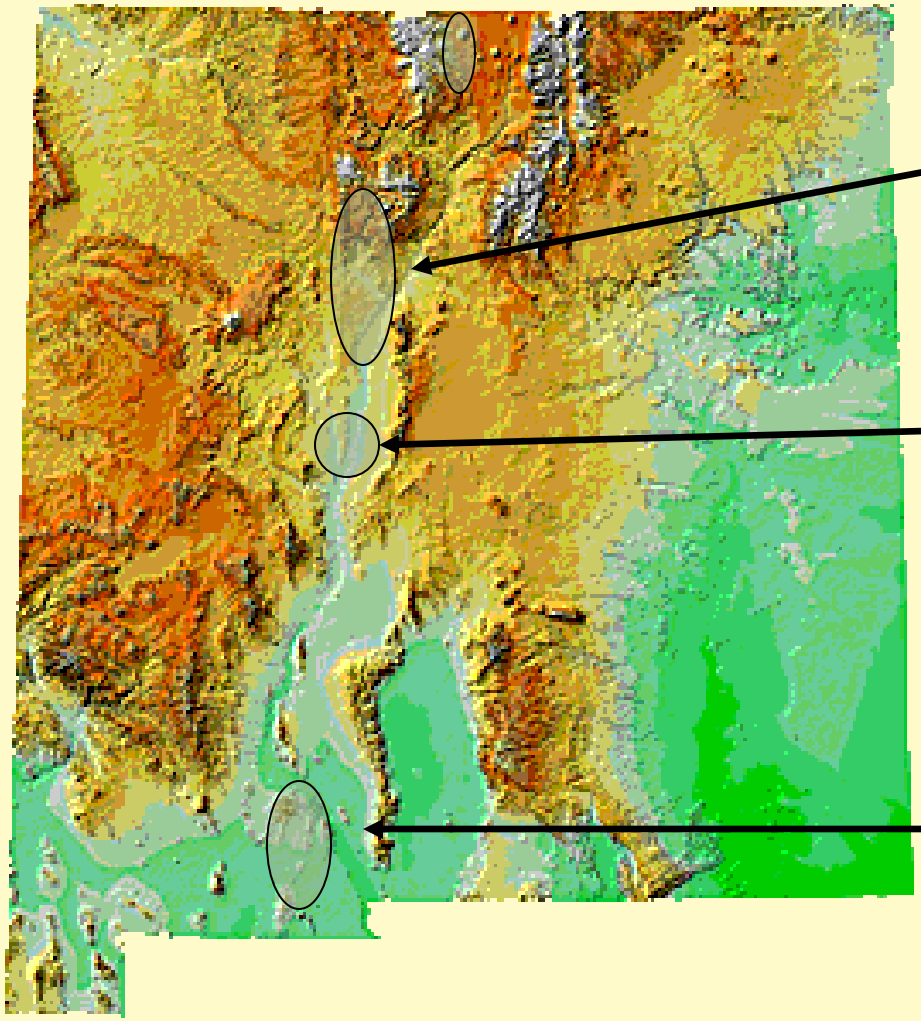
Research question: Do historically and current high As exposures via drinking water contribute to UV-induced melanoma initiation through DNA repair inhibition and genetic susceptibility in a NM non-Hispanic White population?

DNA repair and Cancer

- DNA damage or mistakes in DNA replication are very common.
  - Strand breaks
  - Translocations
- Efficient mechanisms for DNA repair
  - Nucleotide and base excision repair
  - Involve teams of proteins
- Unrepaired damage can lead to cancer
- Co-carcinogens may inhibit DNA repair mechanism
- Interaction studied in this work:
  - UV damages DNA
  - Arsenic inhibits DNA repair



Geochemical assessment for As in New Mexico



- **Abundant in silicic volcanics**
  - **derived volcaniclastic sediments and associated hydrothermal systems**

- **Arsenic enrichment by Potassium Metasomatism**
  - **low temperature alteration common in closed hydrographic basins in arid climates**

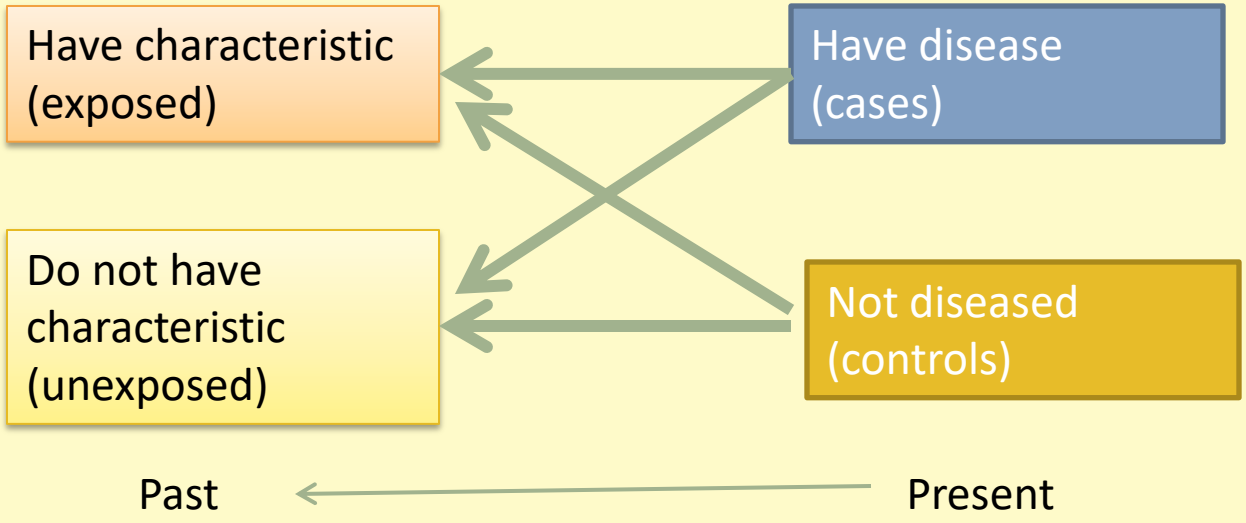
- **Mixing of deep geothermal waters and shallower surface influenced waters**

Controversy over Arsenic MCL

- US EPA Standard Maximum Contaminant Level = 10 μg/L as of 2006
- State MCLs and Public Health Goals are lower
  - NJ MCL = 5 μg/L , CA PHG = 4 μg/L /L; MCLG = 0 μg/L
- Poor health data at lower As concentrations
  - What is shape of dose-response at lower concentrations?
  - Potential use of internal biomarkers to detect internal exposures and effects at lower As concentrations?
- If UV x As interaction important, then need to lower standard?
- Cost of compliance with lower standards*
  - National Annualized cost — New Mexico
    - 10 μg/L : $195M -$495M — $49M-$60M
    - 5 μg/L : $442M - $1,460M — $139M - $172M
  - Unintended consequences: Potential accidents and other loss of life by economic trade-offs could offset potential health benefits.

*Bitner et al. 2001. NM Geology, 10-12.

Case-control study design

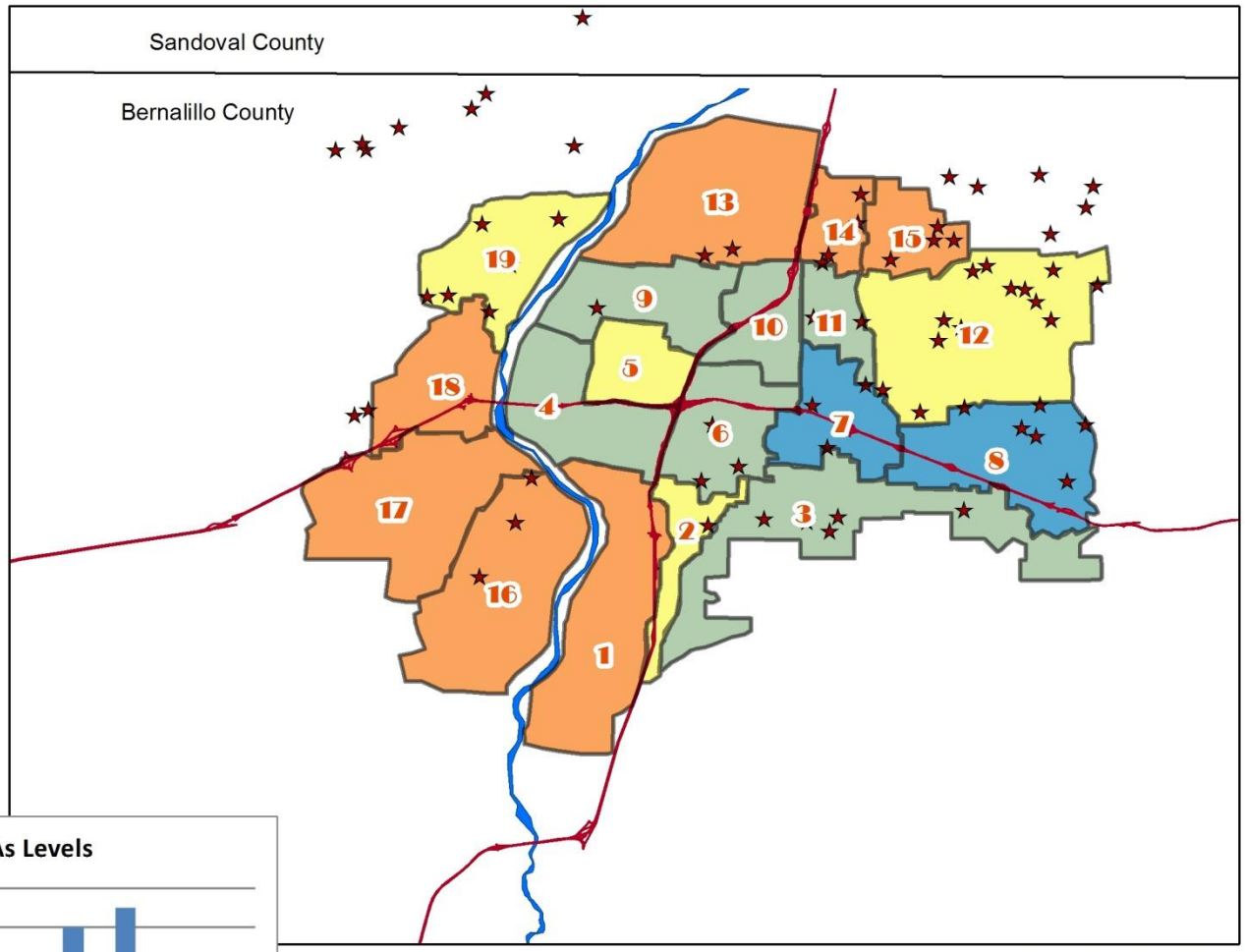


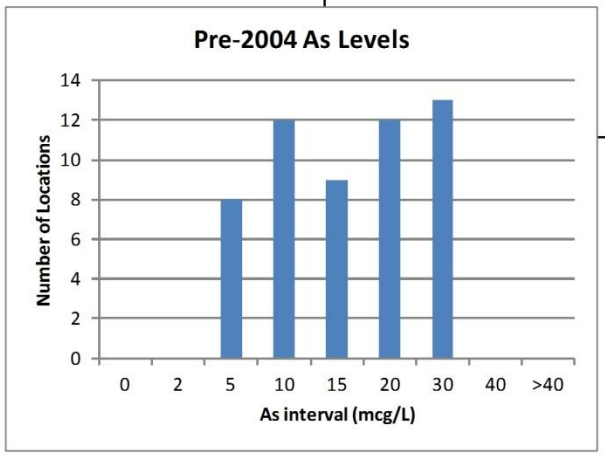
- Retrospective (historical) studies)
- Procedure:
  - Identify **cases** of disease
  - Identify **controls** who are comparable to cases but lack the disease
  - Compare historical exposures of cases and controls to estimate risk to have disease
- Advantages: quick, small size; good for rare disease, low cost
- Disadvantages: biases from case or control selection, information bias

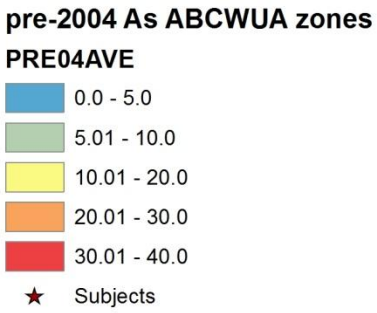
UNM Pilot observational study methods

- Subjects
  - Cases – melanoma cases from NMTR (n = 64)
  - Controls – histologically confirmed benign nevi (n = 35)
- Questionnaires (health & residential histories)
  - Measure current and estimate historic arsenic exposure from databases compiled by USEPA, EWG, NRDC, utilities
    - Drinking water arsenic exposure index calculated by averaging estimated water concentrations for each year/decade of life to age of melanoma diagnosis (cases) or benign nevi removal (controls).
  - Assess current and historic UV exposure by decade of life
    - Estimation of annual ground level UVA, UVB, and erythemal UV (kJ/m^2) adjusted for cloud cover.
- Exposure markers:
  - Current drinking water [iAs] (As^V, As^{III})
  - Urinary iAs, MMA, DMA ($T_{1/2}$ ~ 30 hr)
  - Toenail clipping [total As] (~ 3 months)
  - DNA strand breakage & repair in lymphocytes– Comet assay
  - Genotyping for selected DNA repair genes -- ascertain distribution of genetic polymorphisms

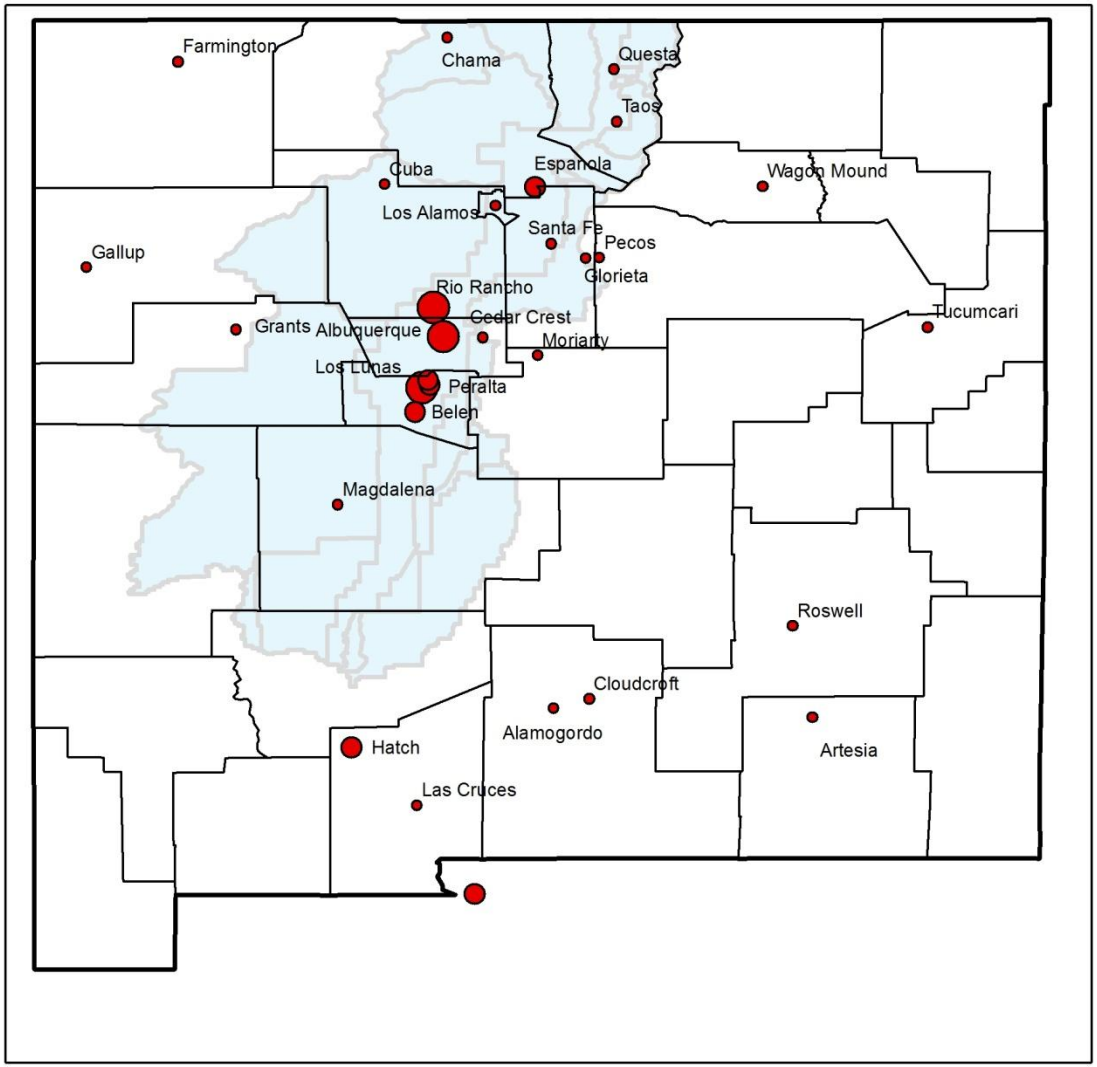
Average ABCWUA Zone Arsenic Concentration (Pre-2004)







Average NM City Arsenic Concentrations (pre-2001)

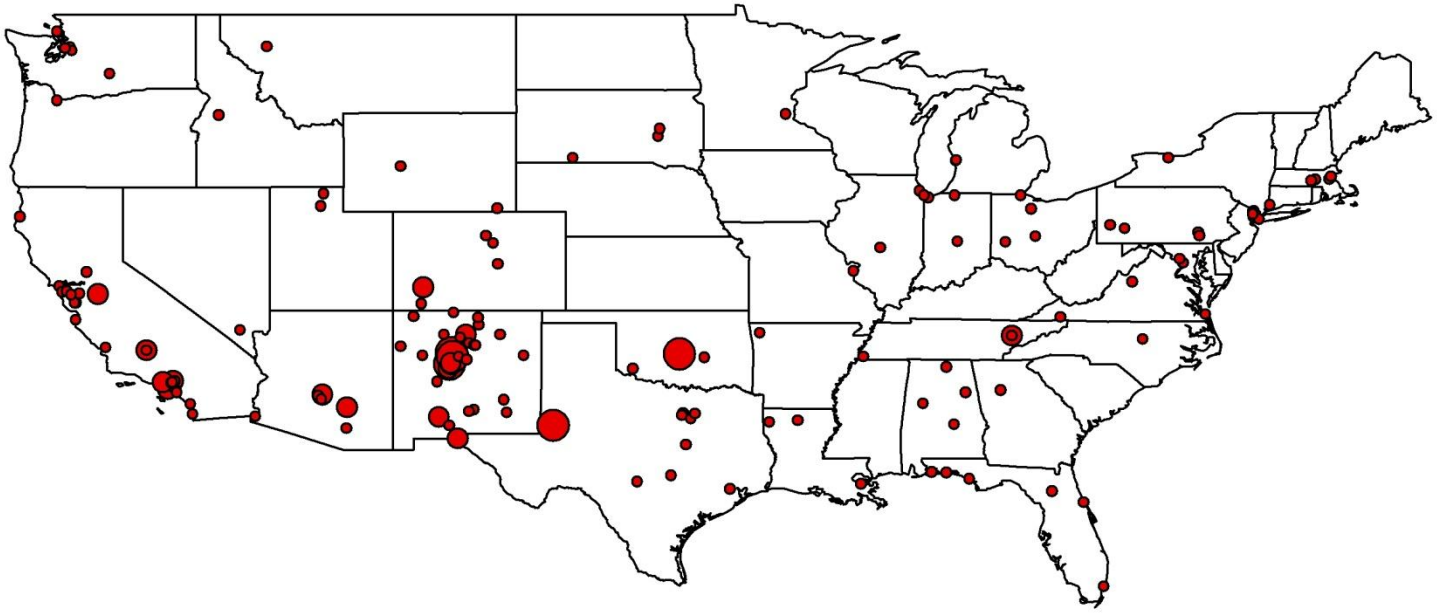


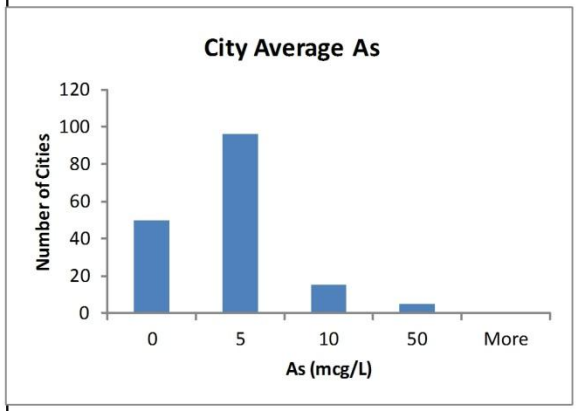
pre-2001 City Average As Concentration

AVEPRE2001

- 0.0 - 5.0
- 5.01 - 10.00
- 10.01 - 50.0
- >50.0
- Rio Grande ground water basin

Average Arsenic Concentrations (pre-2001)





pre-2001 City Average As Concentration
AVEPRE2001
0.0 - 5.0
5.01 - 10.00
10.01 - 50.0
>50.0

Exposure assessment results

- Relationship between As exposure and melanoma
  - **No differences** in drinking water iAs concentrations were observed for public or private water sources between cases and controls.
  - Assessment of individual historical arsenic exposure showed **no significant differences** between cases and controls ($p = 0.09$).
- Relationship between UV exposure and melanoma
  - Mean UV exposure in the 90 days prior to diagnosis of melanoma (cases) or benign nevus (controls) was **significantly higher** for cases (4.20 $kJ/m^2/day \pm 1.22$) than controls (3.46 $kJ/m^2/day \pm 1.16$, $p = 0.001$).

Biomarkers - results

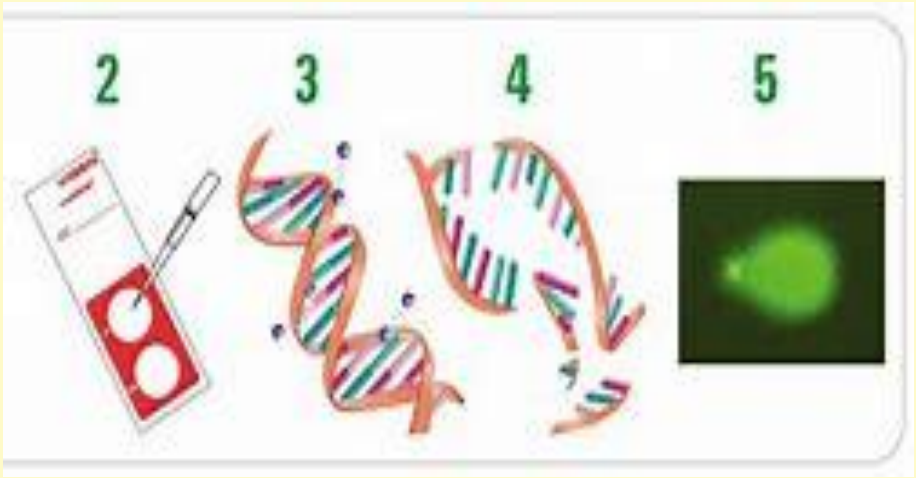
- Differences in biomarkers between cases and controls were **not significant**.
- Relationship between current As exposure and biomarkers in urine and toe nails were highly **significant** $p<0.0001$ for **all** subjects.

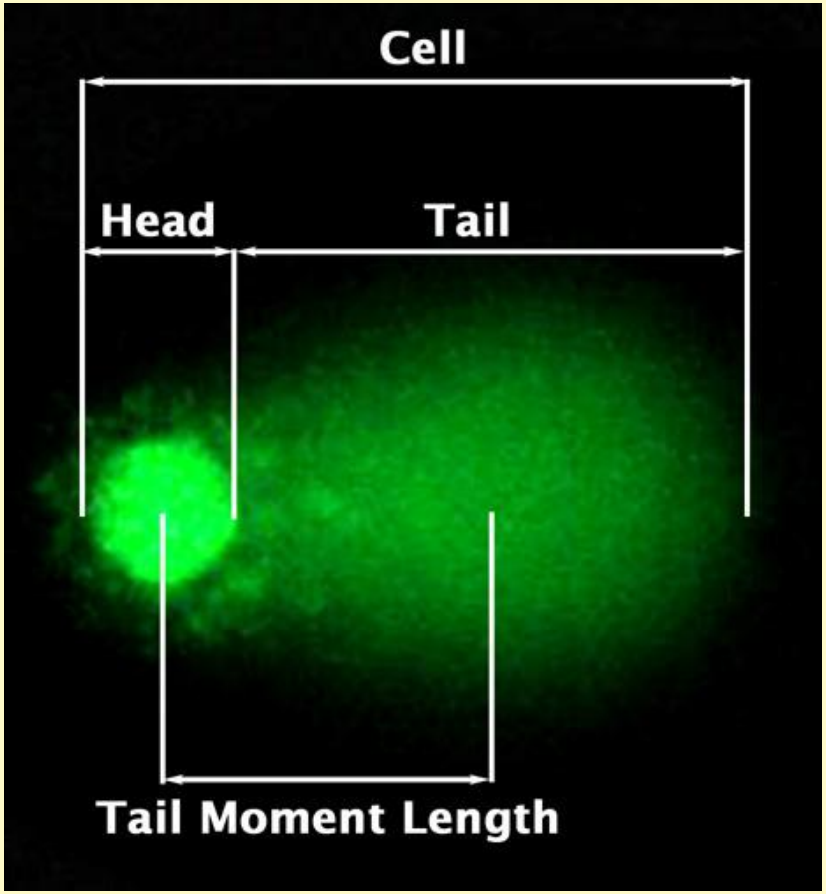
Biomarker	iAs urine	MMA urine	DMA urine	tAs toenail
Pearson r	0.62	0.46	0.50	0.49

- Toenail As concentration was also significantly correlated with the major urinary As species.
- Results indicate a consistent ability to quantify internal exposure to these very low levels of environmental exposure to iAs in drinking water.

Comet assay procedure to measure DNA damage

1. Draw blood.
2. Mount and lyse cells
3. Denature DNA.
4. Apply electric field to separate damaged fragments from intact DNA.
5. Use dyes to visualize DNA.
6. Measure head and tail of ‘comet’ pattern,



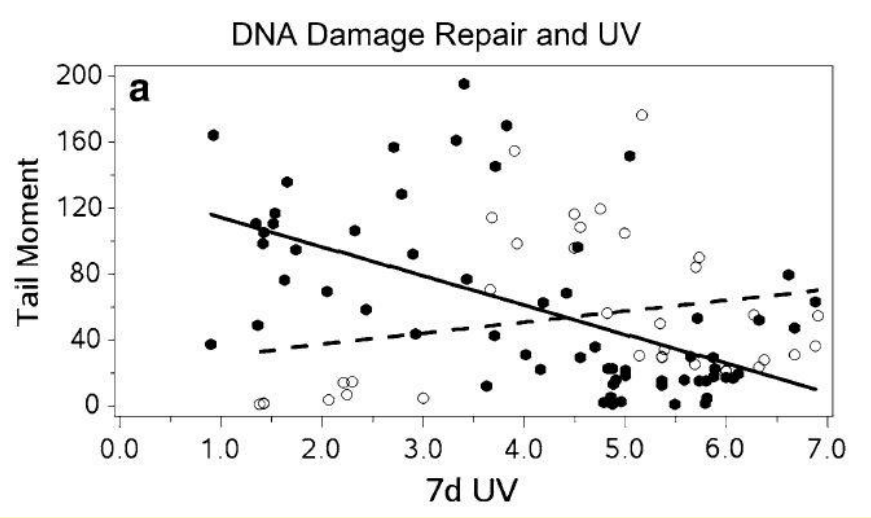


Picture credits: Trevigen Co. website, blood draw BD Biosciences; Comet assay kit produced and is patented to Trevigen Co.Gaithersburg, MD

Extent of DNA liberated from the head of the comet is directly proportional to the amount of DNA damage.

Comet Assay - results

- iAs concentrations in drinking water and internal biomarkers of iAs exposure were **not significantly correlated** with either of the two Comet assay endpoints (%DNA in tail and Tail moment) when controlling for UV exposure.
  - **Little**, if any, in vivo **effect of arsenic exposure on DNA repair** inhibition at these low As drinking water exposure levels
    - Cases: 3.98 µg/L (± 3.67) vs. Controls: 3.47 µg/L (± 2.40)
- However, at low UV values, cases have more DNA damage than controls(i.e. more sensitive to low-level UV exposure)



- Tail moment = tail length x fraction DNA in tail
- 7d UV = UV erythemal previous 7 days (kJ/m^2/day)
- Open circles- controls
- Closed circles – cases

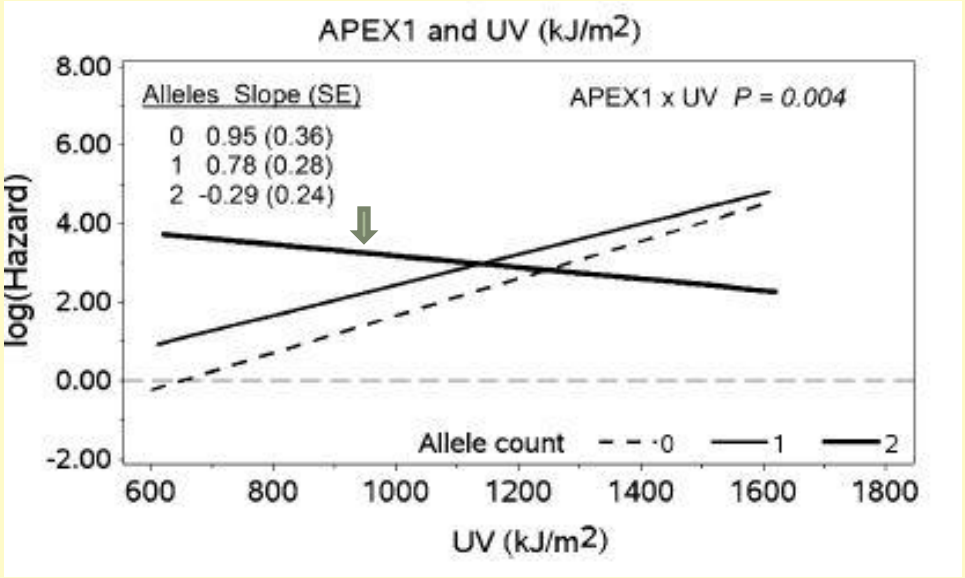
- Slopes are significantly different ($p < 0.001$)

Genetic effects: Single Nucleotide Polymorphisms (SNPs)

- Variation in DNA sequence
  - Substitution, insertion, deletion of base pair
  - >1% frequency in general population
  - 1 SNP every 300-1000 base pairs
- Functional and nonfunctional effect on protein function
  - No effect, increased risk, or protective
  - >60 DNA repair genes
  - Range from 3 to >260 polymorphisms/gene
  - Analysis for DNA repair genes XPD312, XRCC1, APEX1, PARP1, ERCC2 in this study
    - These are common DNA repair genes for which variant frequencies among Caucasians are known to be above 11%
- PCR (polymerase chain reaction) can produce a billion copies of a gene in 30 cycles.

SNP analysis results

- Specific SNPs not significantly associated with melanoma in this population.
  - No difference between cases and controls in mean % of SNPs for any gene
- APEX1 x time-variable UV exposure interaction was observed (p = 0.004).
  - APEX1 (rs 1130409) – DNA base excision repair gene with 3 genotypes
  - UV exposure: from age 1 to age at first melanoma diagnosis
  - Individuals with 2 copies of SNP (homozygous) had higher risk for melanoma at low exposures than other genotypes.



Alleles
0 = wild type (normal)
1 = heterozygous (1 copy)
2 = homozygous (2 copies)

Env Geochem Health (2015)
(DOI 10.1007/s10653-09770-4

Conclusions

- Exposure to drinking water As, either alone or in conjunction with UV exposure, was not associated with increased hazard of a melanoma diagnosis in this study population
  - At these low As drinking water exposure levels, there may be little, if any, in vivo effect of arsenic exposure on DNA repair inhibition.
  - Consistent with other studies that found no association between arsenic exposures at low (<25 μg/L) levels and a variety of cancers including melanoma.
  - Other population and lab studies at higher As concentrations have found associations with DNA damage and cancers.
- Mean UV exposure was significantly higher for cases.
  - At low UV values, cases have more DNA damage than controls (i.e. more sensitive to low-level UV exposure)
- No difference between cases and controls in mean % of SNPs for any gene.
  - However, individuals with 2 copies of SNP (homozygous) had higher risk at low UV exposures than other genotypes.

- Main weaknesses of study were small sample size and difficulties in estimating historical exposures in “migrant” population.
  - Complex residence history of subjects (149 cities)
  - Public water systems have time-varying mix of groundwater and surface water sources.
  - Poor data on historical data for specific wells used by any given subject
  - Analytical detection limits change over time
- Main strength of study: Use of sensitive biomarkers of exposure and DNA damage provides added confidence that effects at low levels of environmental exposure have been detected.

Acknowledgements

National Institute of Environmental Health Sciences (NIEHS) Grant #1R21ES018705 under direction of Janice Yager PhD and Marianne Berwick PhD of the Department of Internal Medicine, UNM .

•Orrin Meyers PhD of Department of Internal Medicine, UNM - UV exposure study and statistical analysis.
•Karen Cooper, PhD, Laurie Hudson PhD of UNM School of Pharmacy - Comet assays
•Kirsten White PhD and the Berwick Molecular Epidemiology Laboratory - gene polymorphism assays.