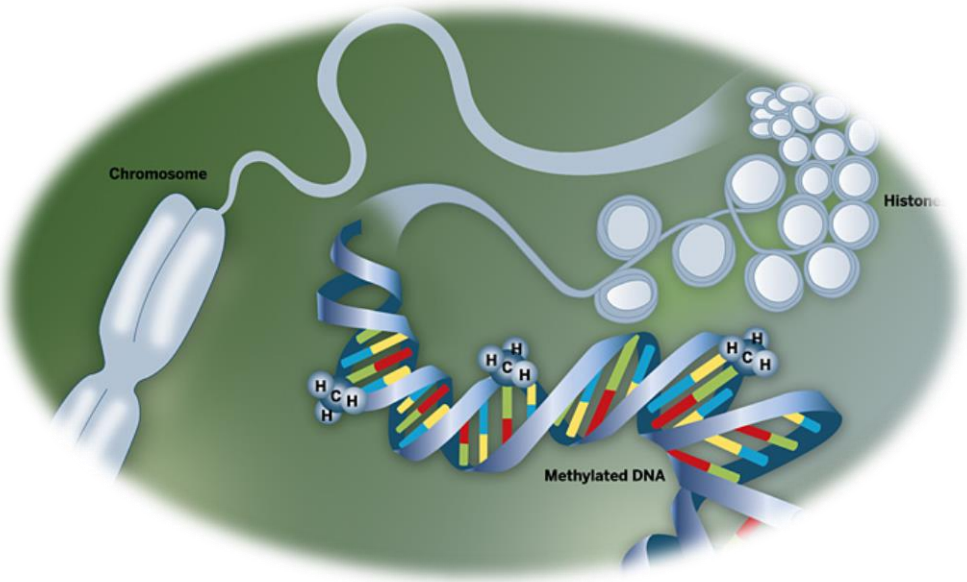
EPA Temporal Exposure Issues for Environmental Pollutants: Prenatal and Early Life Exposure to Arsenic



Rebecca Fry, Ph.D.
Associate Professor, Department of Environmental Sciences and Engineering
Director, UNC SRP-NC-Center for Environmental Risk Analysis
Director, Graduate Studies, Curriculum in Toxicology
Lineberger Comprehensive Cancer Center

National Institute of Environmental Health Sciences
Superfund Research Program

Major questions related to children's environmental health

1. What are the **sources of metal exposure** to in infants and children?

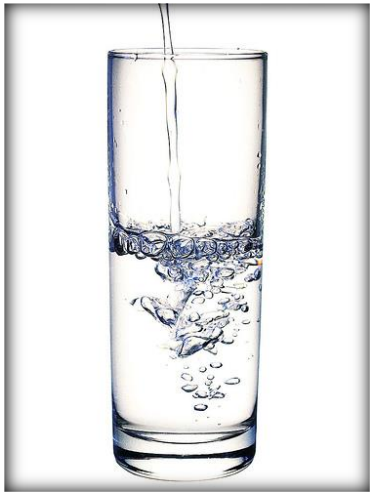
2. What are the **health effects and underlying biological mechanisms**?

3. Can we **predict** who is most at risk?

4. Are there **ways to protect** children from the detrimental health effects of these exposures?

There are various potential sources of exposure for children: locally and globally

Drinking water

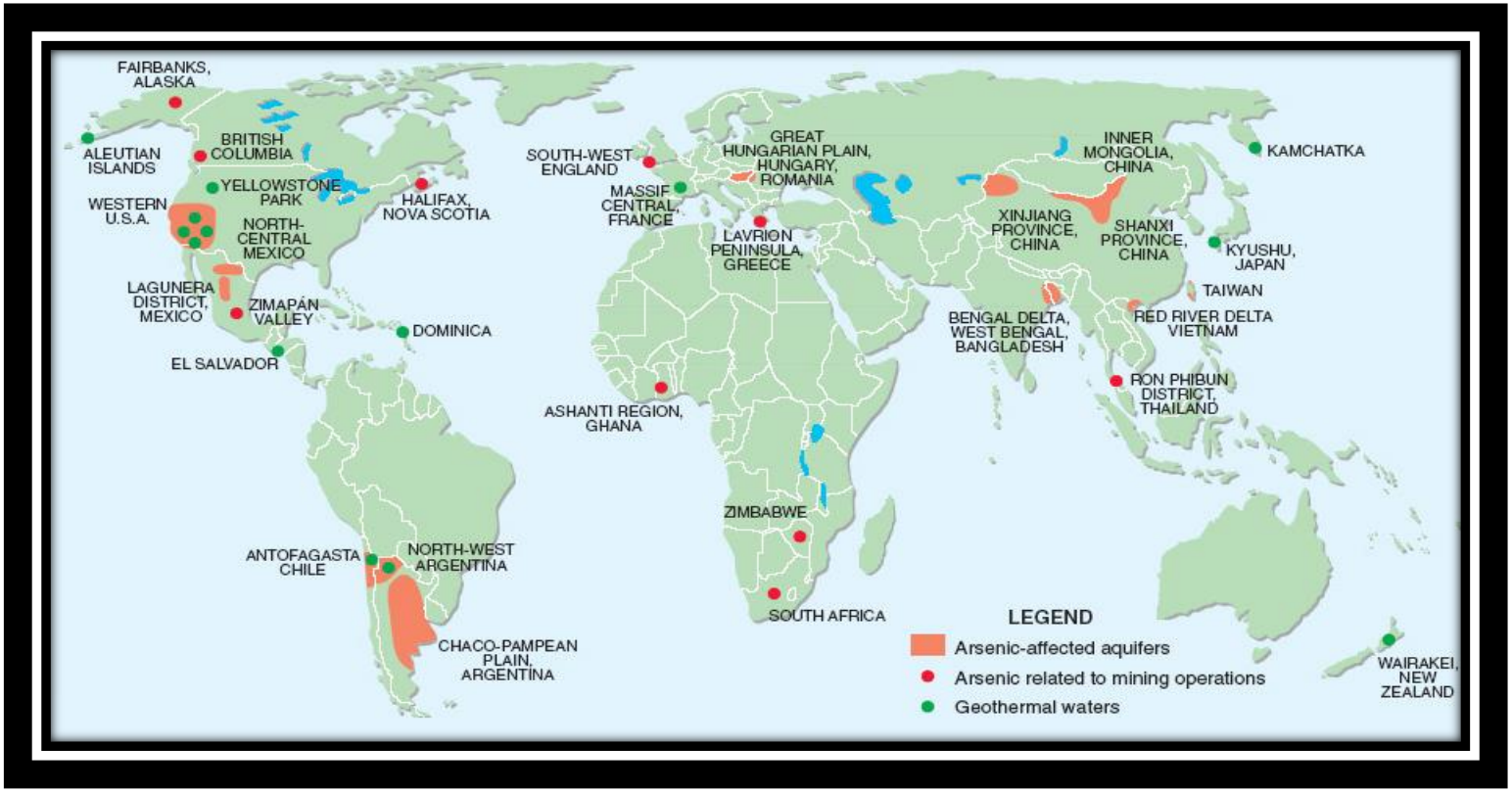


Contaminated food

Transplacental

What are the relationships among levels of inorganic arsenic, timing of exposure, and health outcomes in **humans**?

Arsenic: continues to poison the water of millions around the globe



Smedley, Pauline L.. 2008 Sources and distribution of arsenic in groundwater and aquifers. In: Appelo, Tony, (ed.) *Arsenic in Groundwater : a World Problem.* British Geological Survey
EPA limit 10 ppb

Arsenic: continues to poison the water of millions around the globe

A Sluggish Response to Humanity's Biggest Mass Poisoning

Arsenic-laced water has sickened thousands in South Asia. After delays and false starts, India is addressing the problem with a $500 million safe-water initiative

www.sciencemag.org SCIENCE VOL 315 23 MARCH 2007

Arsenic: continues to poison the water of millions around the globe

Smedley, Pauline L.. 2008 Sources and distribution of arsenic in groundwater and aquifers. In: Appelo, Tony, (ed.) *Arsenic in Groundwater : a World Problem.* British Geological Survey
EPA limit 10 ppb

Prenatal inorganic arsenic exposure in Ron Pibul Thailand

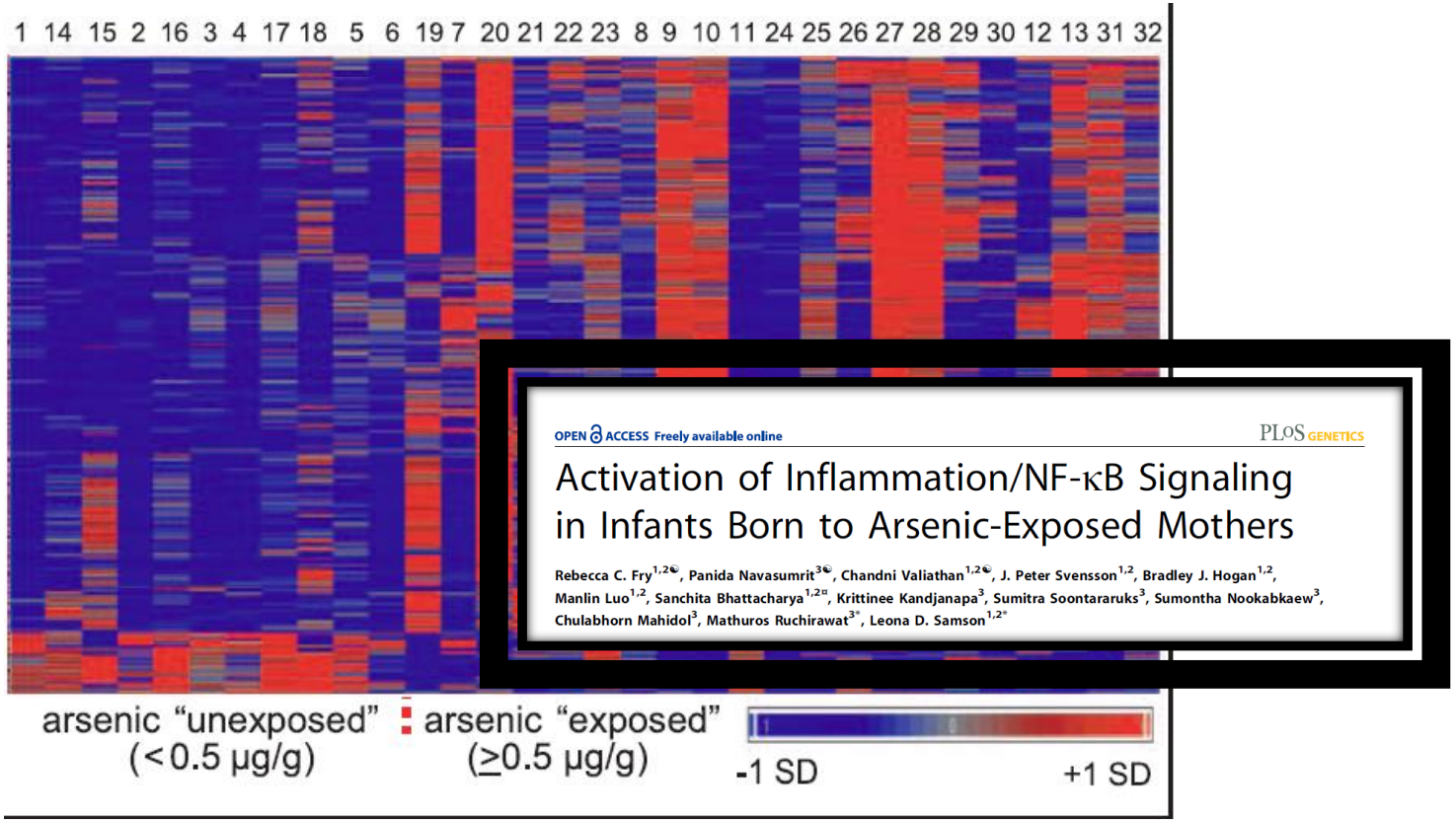
- Population: Ron Pibul and Bangkok, Thailand
- Contamination a result of tin mining activities that took place decades ago

- Water: Drinking water: up to 503.5 µg/L
- 0.1-58.63 µg/g As in mother's toenails
- Prenatal window isolated

Fry, R. C., Navasumrit, P., Valiathan, C., Svensson, J. P…Samson, L. Activation of inflammation/NF-kappaB signaling in infants born to arsenic-exposed mothers. *PLoS genetics, 3*(11), e207.

Massive genomic dysregulation identified

~500 genes identified with altered expression



OPEN ACCESS Freely available online

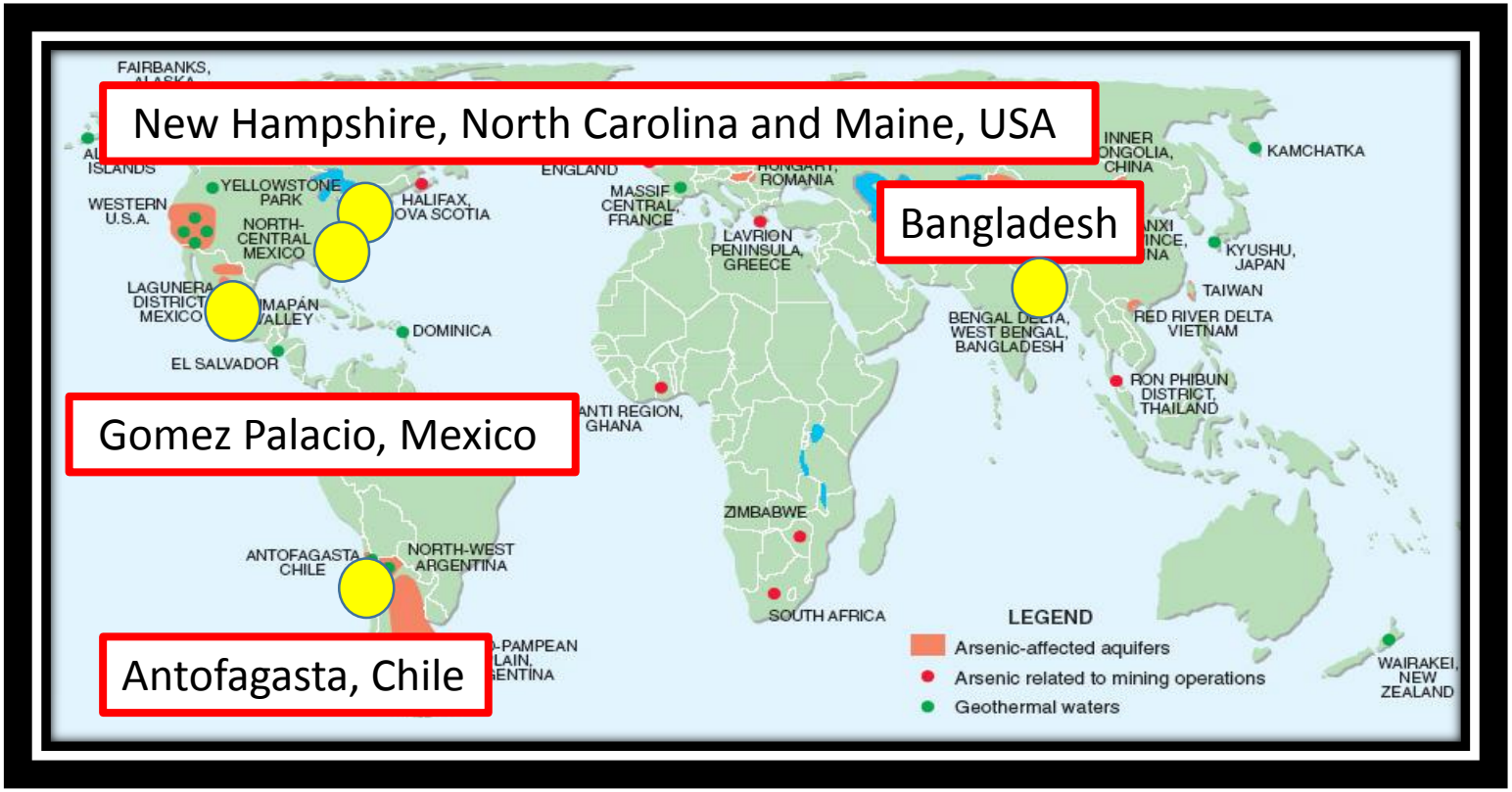
PLoS GENETICS

Activation of Inflammation/NF-κB Signaling in Infants Born to Arsenic-Exposed Mothers

Rebecca C. Fry[1,2☯], Panida Navasumrit[3☯], Chandni Valiathan[1,2☯], J. Peter Svensson[1,2], Bradley J. Hogan[1,2], Manlin Luo[1,2], Sanchita Bhattacharya[1,2¤], Krittinee Kandjanapa[3], Sumitra Soontararuks[3], Sumontha Nookabkaew[3], Chulabhorn Mahidol[3], Mathuros Ruchirawat[3*], Leona D. Samson[1,2*]

What controls this response?
What are long term effects of these exposures?

Arsenic: continues to poison the water of millions around the globe



Smedley, Pauline L.. 2008 Sources and distribution of arsenic in groundwater and aquifers. In: Appelo, Tony, (ed.) *Arsenic in Groundwater : a World Problem.* British Geological Survey
EPA limit 10 ppb

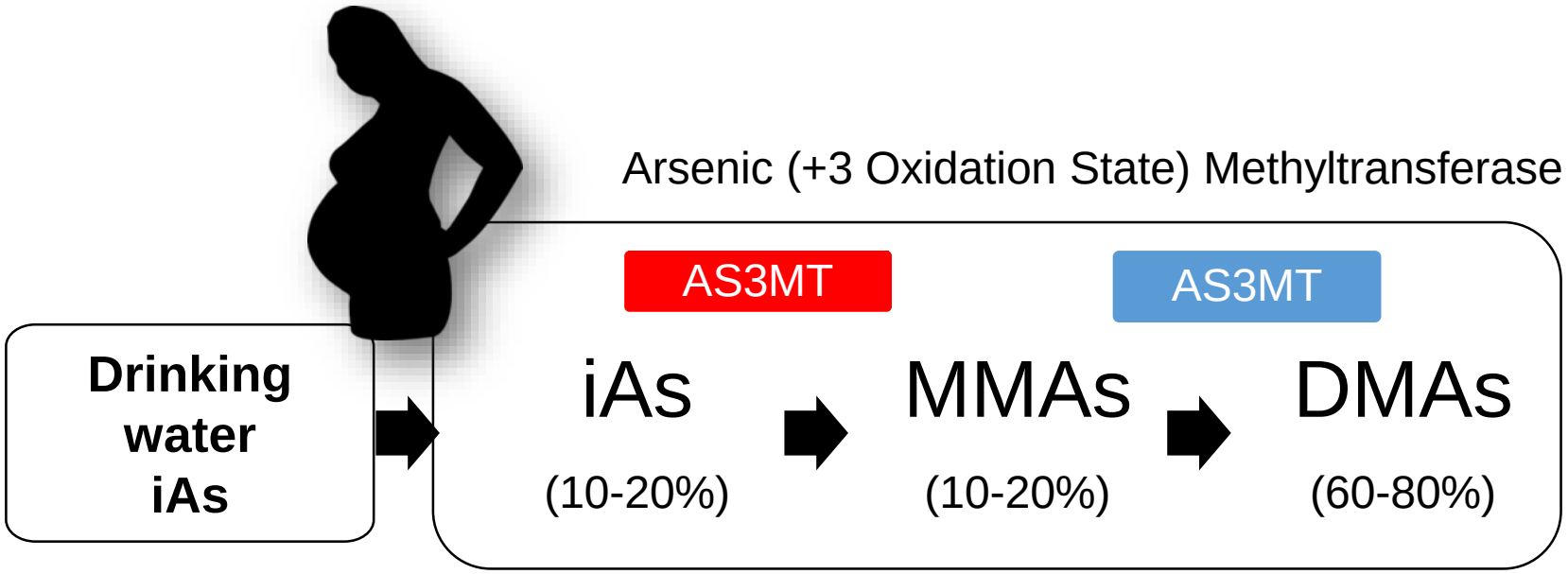
Early life exposure to inorganic arsenic in Gomez Palacio, Mexico

- Contamination a result of naturally occurring inorganic arsenic
- Water had been documented as contaminated-no biomonitoring studies
- 200 mother-baby pairs for the BEAR cohort (n=200)
- Water and urine measured for inorganic arsenic and arsenic species

- Drinking water: mean iAs of 24.6 µg/L
- range of <0.46-236 µg/L.
- Prenatal window isolated

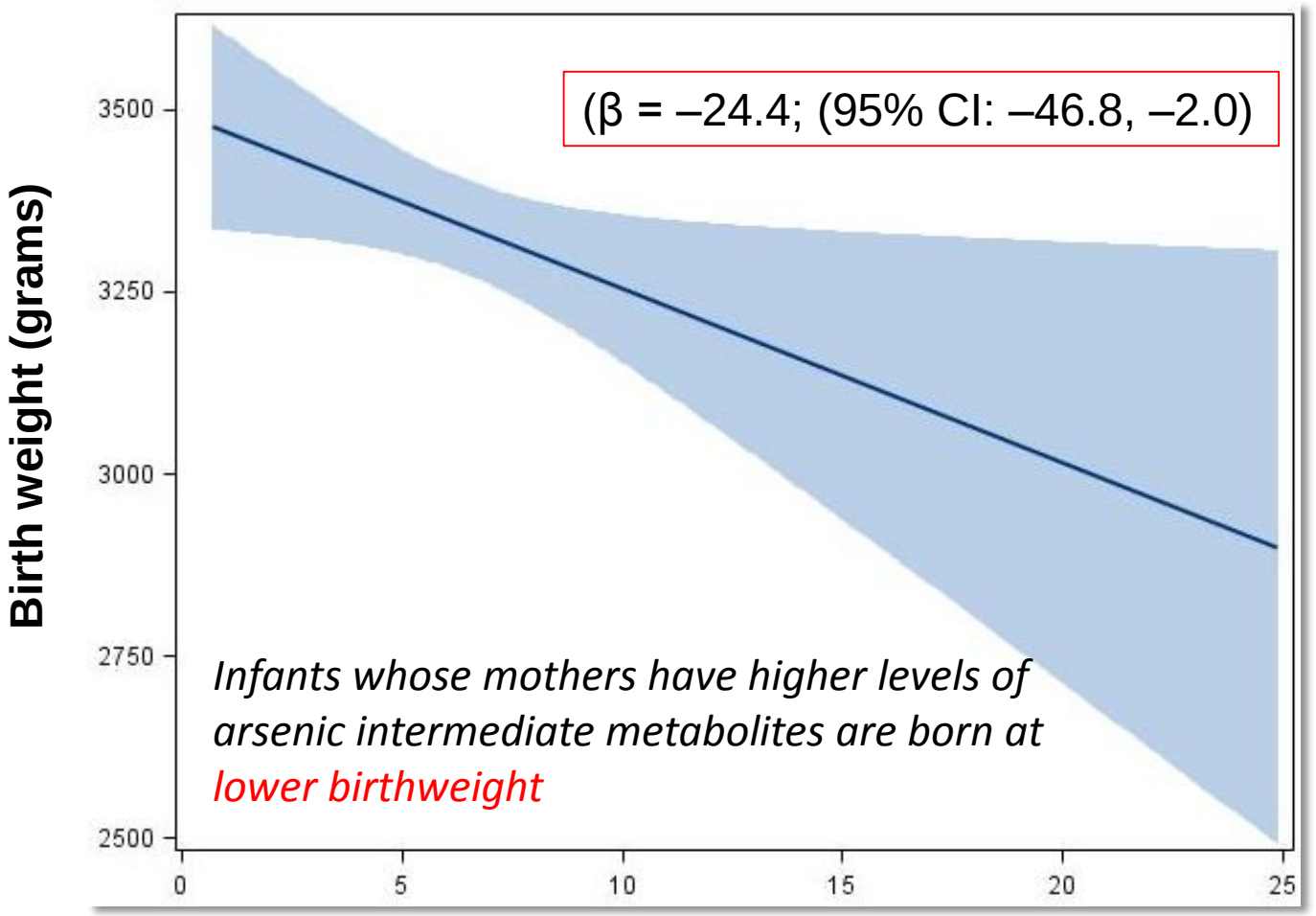
Laine, J. E., Bailey, K. A., Rubio-Andrade, M., Olshan, A. F., Smeester, L., Drobná, Z., . . . Fry, R. C. (2015). Maternal Arsenic Exposure, Arsenic Methylation Efficiency, and Birth Outcomes in the Biomarkers of Exposure to ARsenic (BEAR) Pregnancy Cohort in Mexico. *Environmental health perspectives, 123*(2), 186-192. doi: 10.1289/ehp.1307476

Arsenic metabolism indicators were measured in maternal urine



Efficiency of arsenic metabolism is known to affect susceptibility to arsenic toxicity

Increased levels of MMAs are associated with decreased birth weight in infants



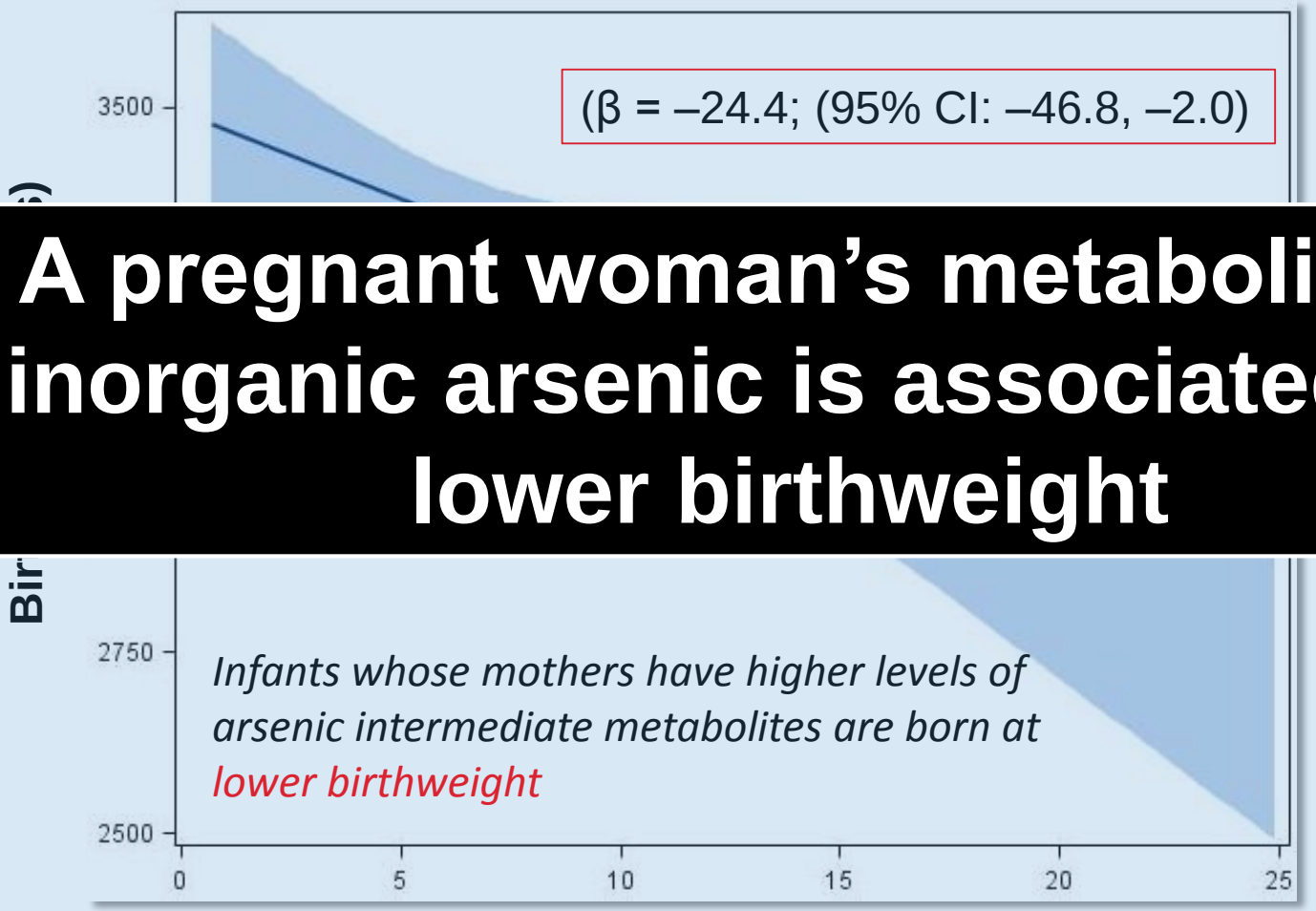
Birth Outcomes:

Gestational age
APGAR score
Newborn length
Head circumference
Small for gestational age
Large for gestational age
Low birthweight
Newborn birthweight

MMAs associated with arsenic toxicity: skin lesions (Ahsan et al. CEBP 2007), bladder cancer, atherosclerosis

ehp ENVIRONMENTAL HEALTH PERSPECTIVES

Increased levels of MMAs are associated with decreased birth weight in infants



Birth Outcomes:
Gestational age
APGAR score
Newborn length
Head circumference

A pregnant woman's metabolism of inorganic arsenic is associated with lower birthweight

MMAs associated with arsenic toxicity: skin lesions (Ahsan et al. CEBP 2007), bladder cancer, atherosclerosis

ehp ENVIRONMENTAL HEALTH PERSPECTIVES

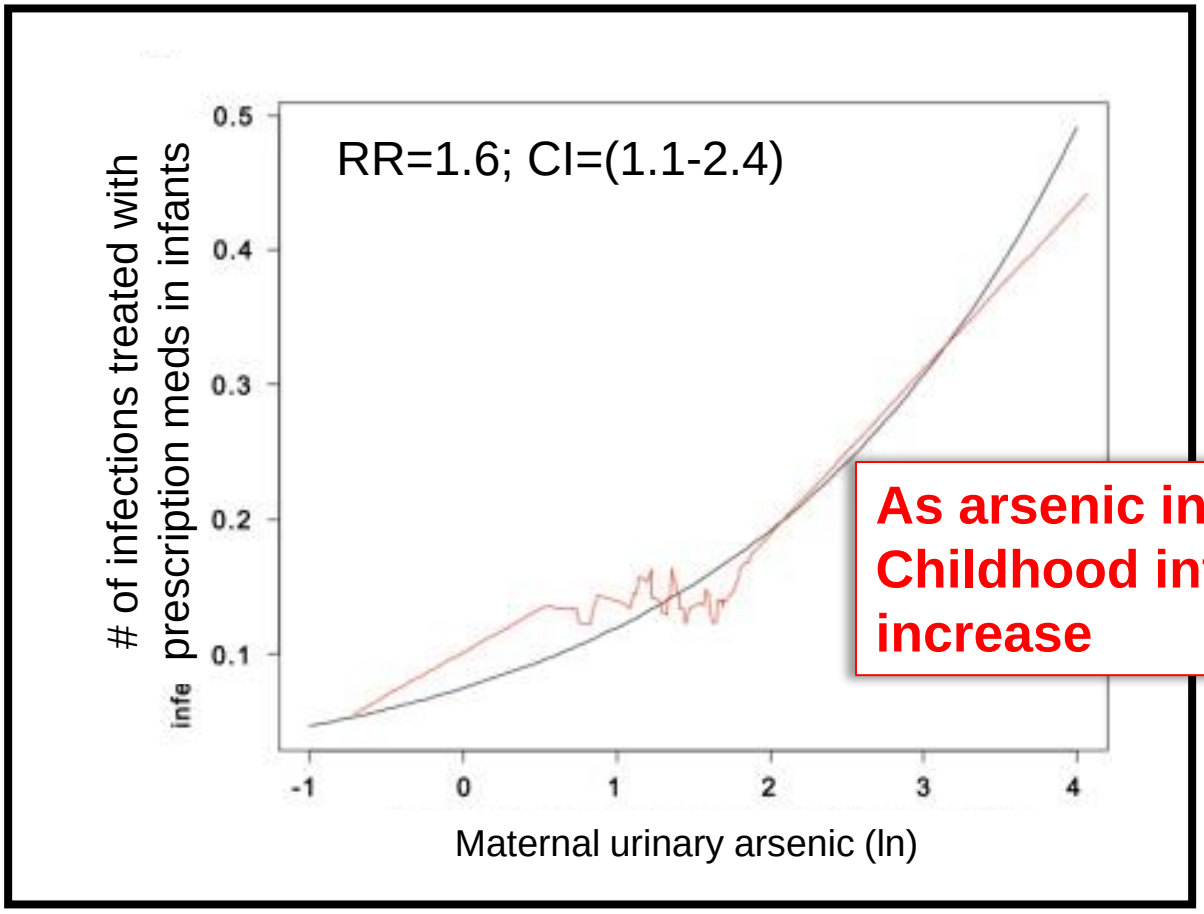
Prenatal and early life arsenic exposure in New Hampshire

- Contamination a result of naturally occurring inorganic arsenic
- New Hampshire Birth Cohort (n=214)

- Drinking water with a mean of 5.2 µg/L and a range of 0.01-67.5 µg/L.
- Maternal urinary arsenic levels mean of 6.0 µg/L, range of 0.45-58.3 µg/L.
- Prenatal and early life exposure and infectious measured at 4 months of age

Farzan, S. F., Korrick, S., Li, Z., Enelow, R., Gandolfi, A. J., Madan, J., . . . Karagas, M. R. (2013). In utero arsenic exposure and infant infection in a United States cohort: A prospective study. *Environ Res, 126*, 24-30.

Increased risk of infection associated with prenatal and early life arsenic exposure in New Hampshire

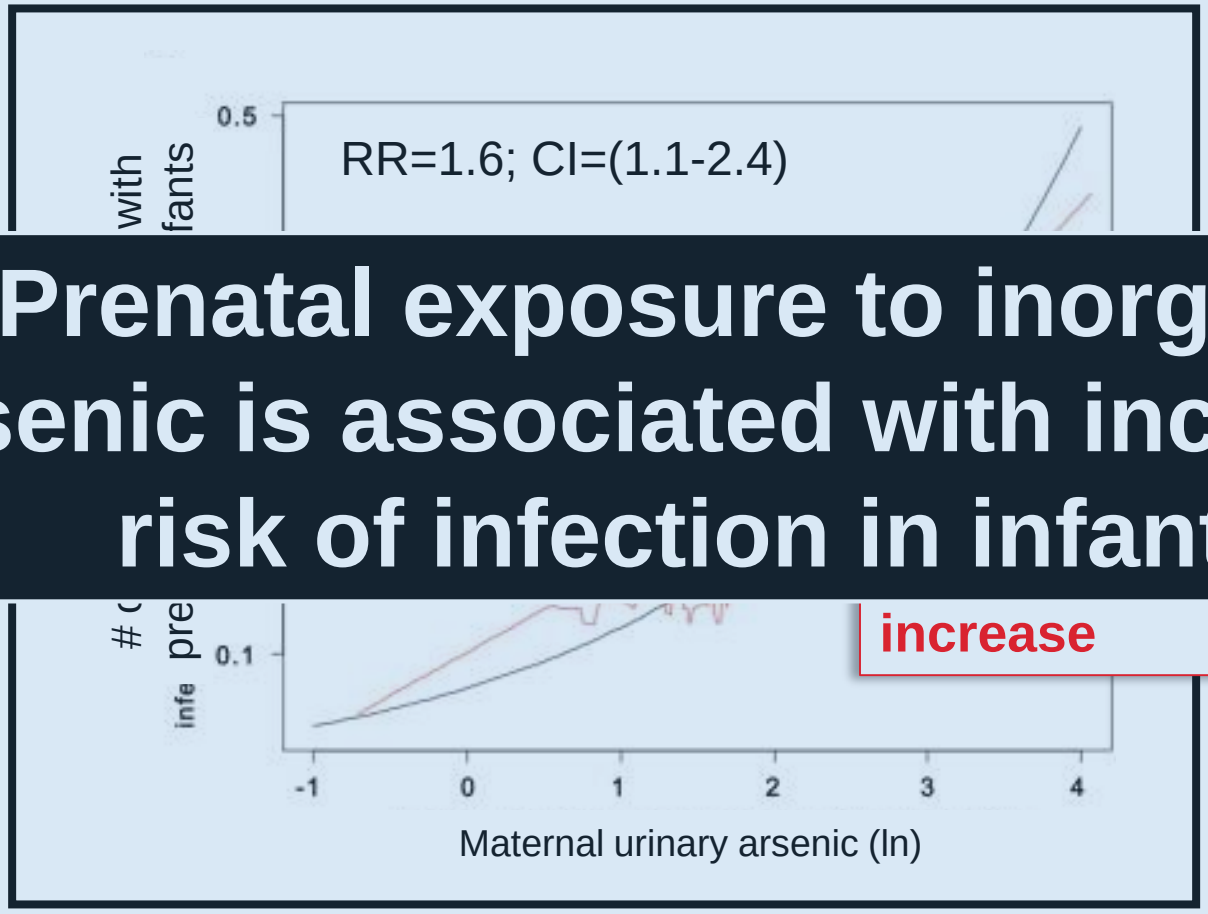


As arsenic increases Childhood infections increase

Respiratory tract infections

Environmental Research 126 (2013) 24–30

Increased risk of infection associated with prenatal and early life arsenic exposure in New Hampshire



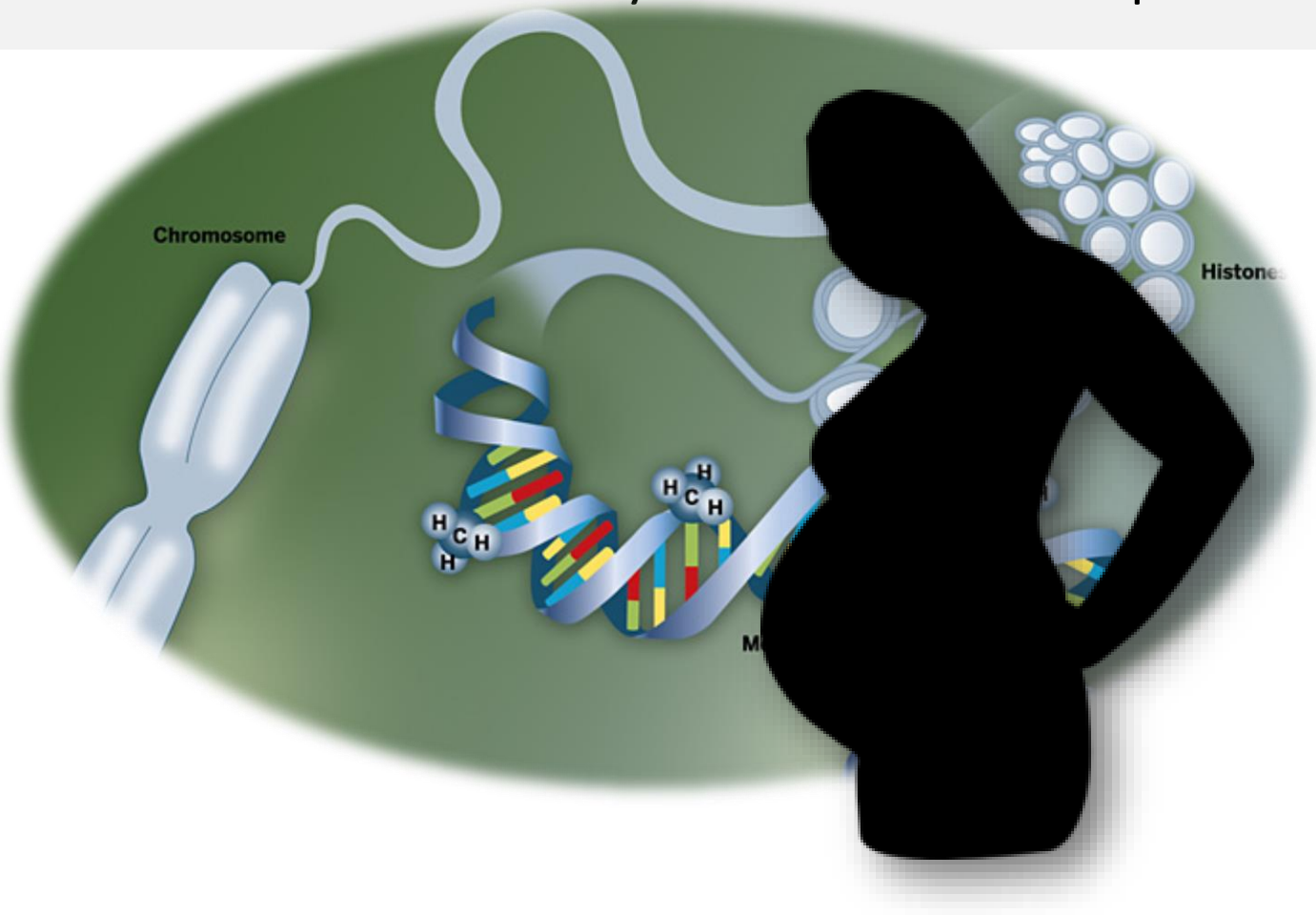
Prenatal exposure to inorganic arsenic is associated with increased risk of infection in infants

increase

Respiratory tract infections

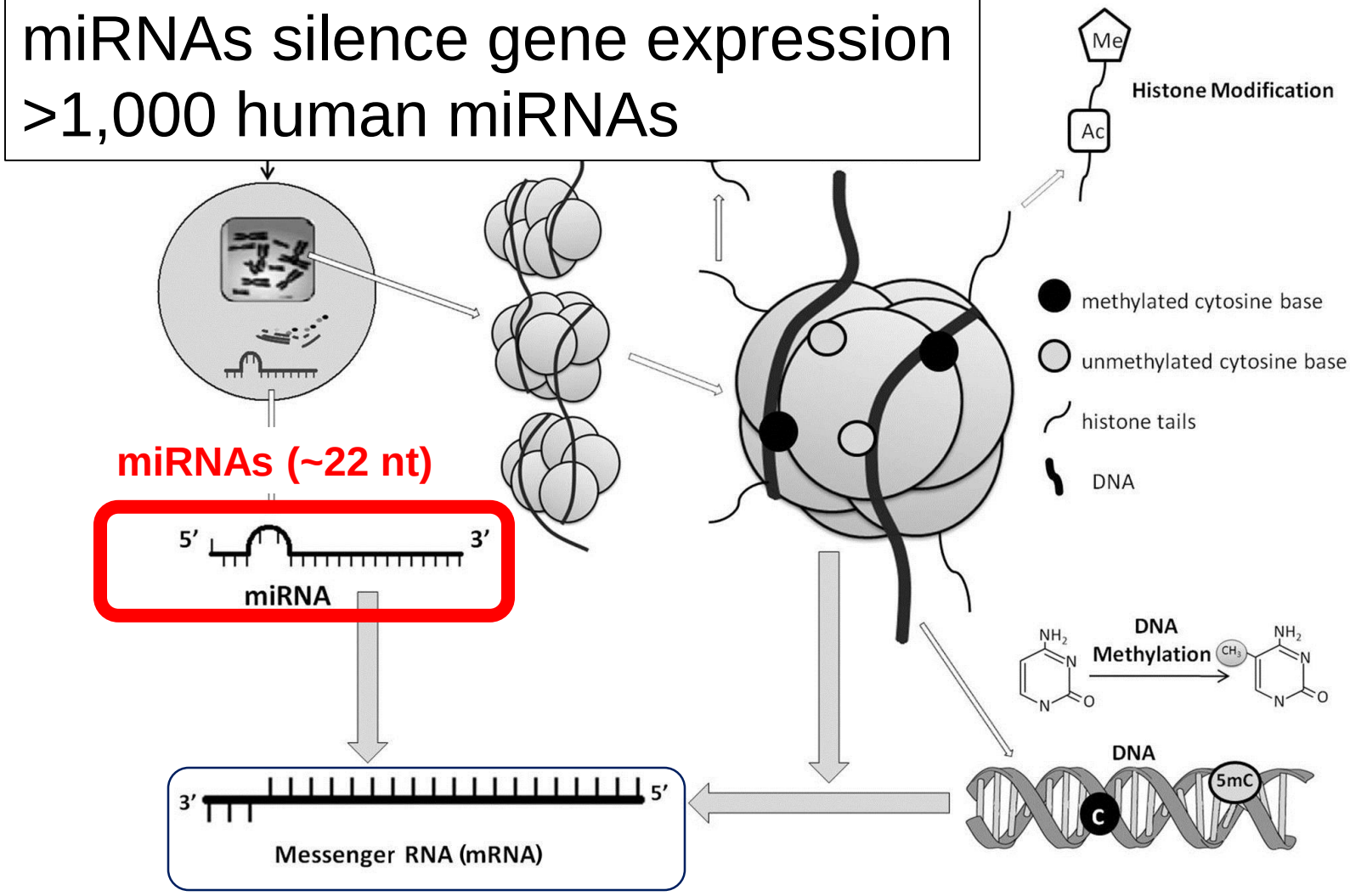
Environmental Research 126 (2013) 24–30

Do epigenetic mechanisms underlie the **increased risk for infections** associated with early life arsenic exposure?



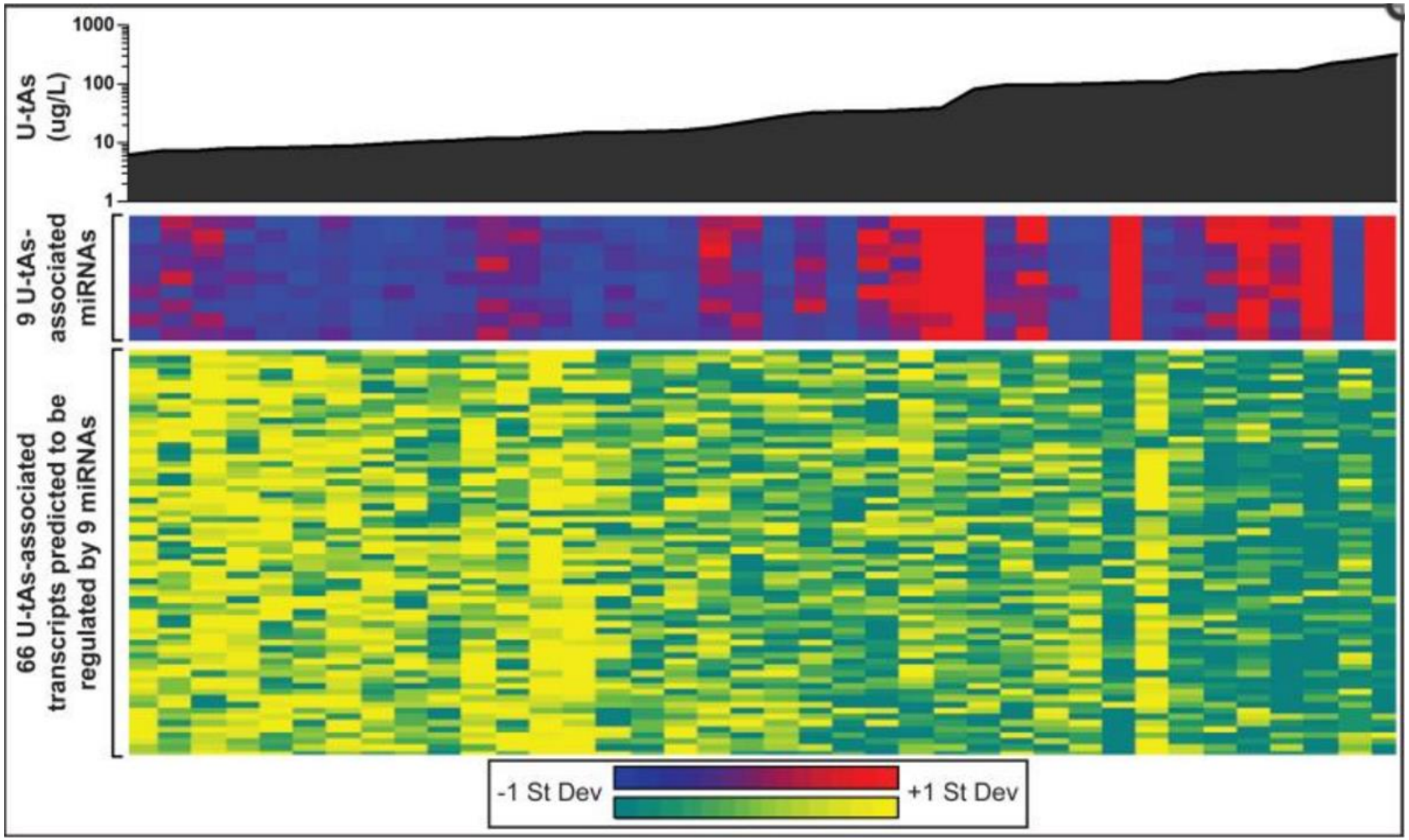
Epigenetic changes influence gene expression

miRNAs silence gene expression
>1,000 human miRNAs

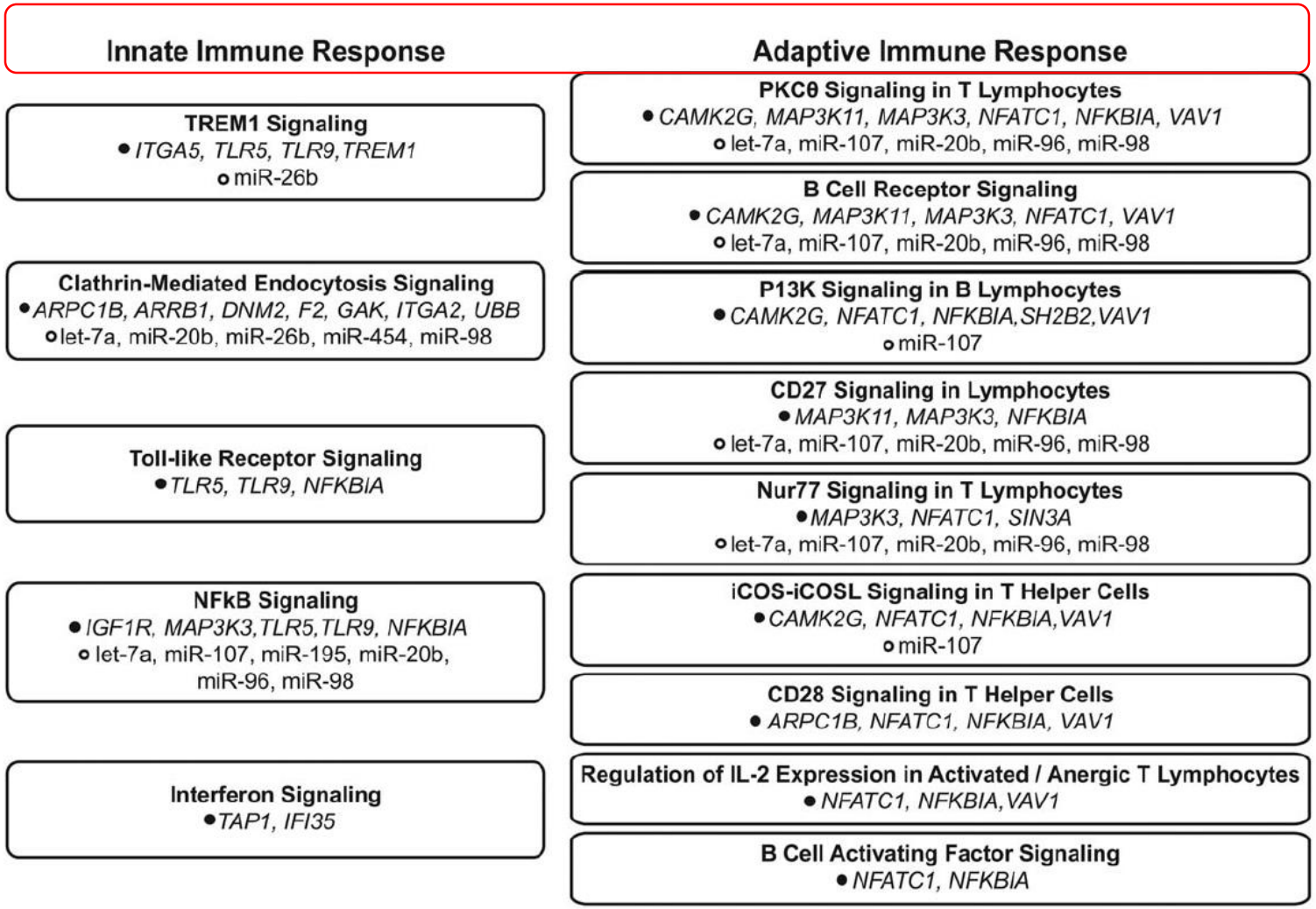


Hou L et al. Int. J. Epidemiol. 2012;41:79-105

Epigenetic factors as biomarkers of inorganic arsenic exposure



miRNAs mediate and suppress the innate and adaptive immune response



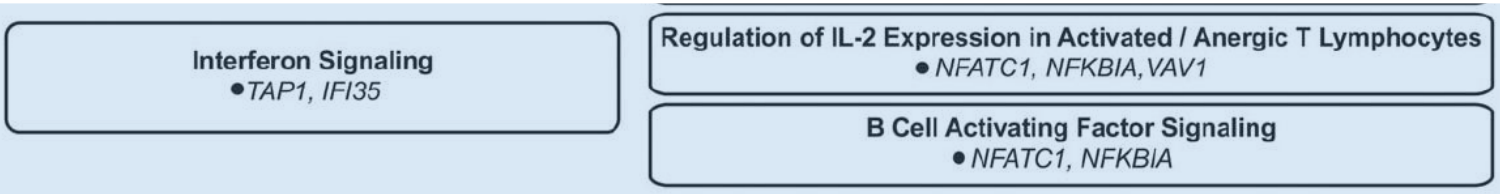
TLR5: response to bacteria

Rager et al 2014

miRNAs mediate and suppress the innate and adaptive immune response

miRNAs REGULATE the expression of innate/adaptive immune response genes

Role in response to infectious agents



TLR5: response to bacteria

Rager et al 2014

frontiers in GENETICS

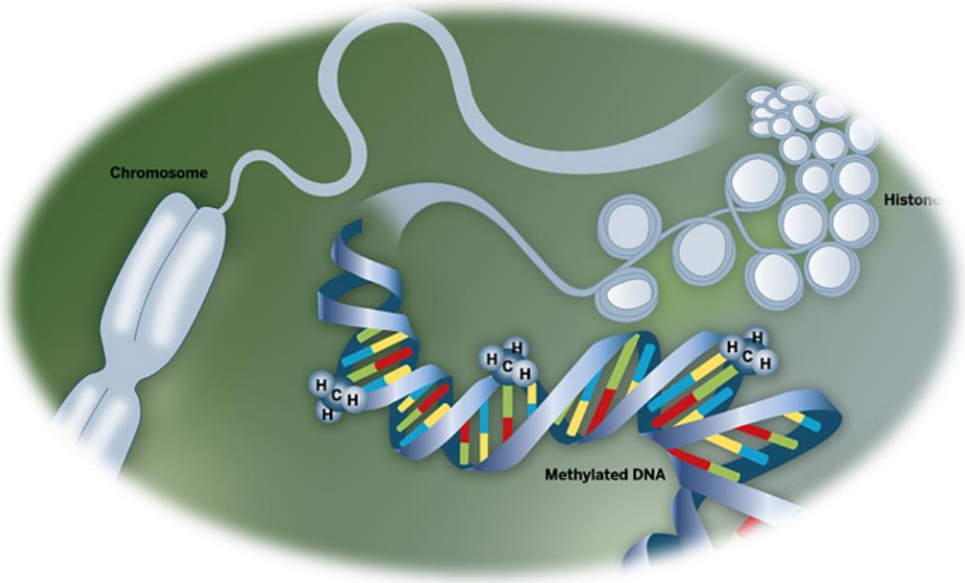
REVIEW ARTICLE
published: 16 July 2014
doi: 10.3389/fgene.2014.00201

Incorporating epigenetic data into the risk assessment process for the toxic metals arsenic, cadmium, chromium, lead, and mercury: strategies and challenges

Paul D. Ray[1,2†], Andrew Yosim[1†] and Rebecca C. Fry[1,2]*

[1] *Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA*
[2] *Curriculum in Toxicology, School of Medicine, University of North Carolina, Chapel Hill, NC, USA*

- Understand molecular mechanisms of disease
- Integration of cellular and molecular biomarkers into the risk assessment process



Prenatal and early life exposure to arsenic in Bangladesh

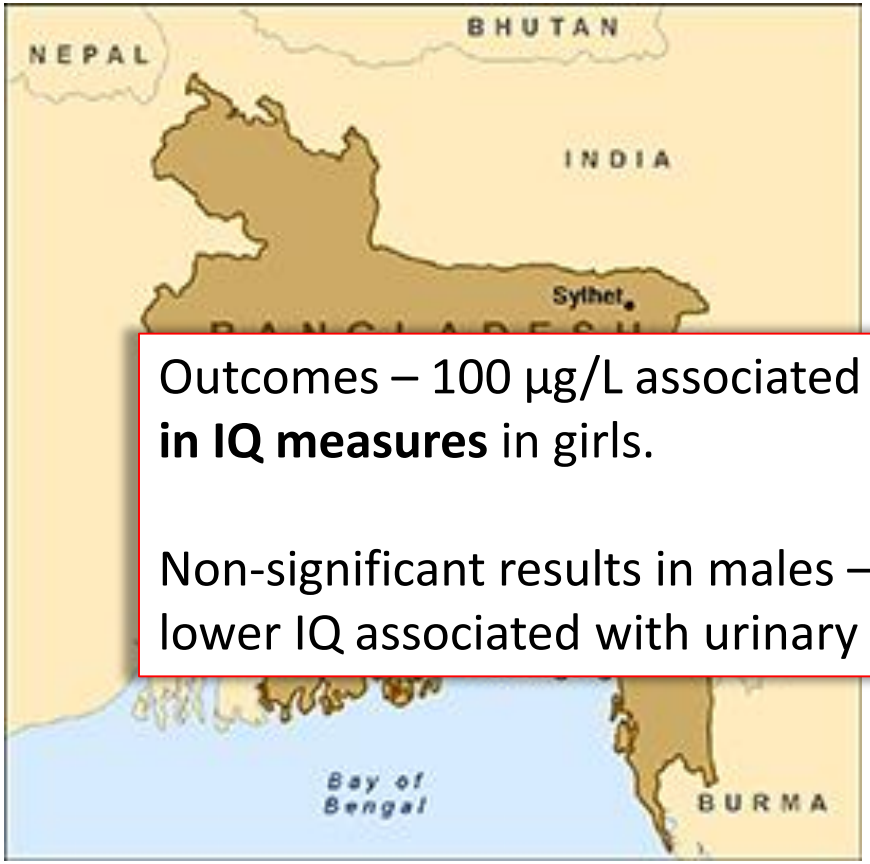
- MINIMat Cohort: Matlab, Bangladesh (n=2200)
- Contamination a result of naturally occurring inorganic arsenic

- Drinking water with median arsenic levels of 66 µg/L and 90th percentile level of 410 µg/L.
- Measurements taken **during pregnancy at 8 and 30 weeks gestation** and in **c**hildren uTAs was measured at **18 mos and 5 years**

- Looked for associations between measures at each of these time points and neurodevelopmental outcomes, unable to limit exposure to just prenatal or early life.

Hamadani, J. D., Tofail, F., Nermell, B., Gardner, R., Shiraji, S., Bottai, M., . . . Vahter, M. (2011). Critical windows of exposure for arsenic-associated impairment of cognitive function in pre-school girls and boys: a population-based cohort study. *Int J Epidemiol, 40*(6), 1593-1604. doi: 10.1093/ije/dyr176

Prenatal and early life exposure to arsenic in Bangladesh



Outcomes – 100 µg/L associated with a **1-3 point decrease in IQ measures** in girls.

Non-significant results in males – trend showed consistently lower IQ associated with urinary arsenic.

- MINIMat Cohort: Matlab, Bangladesh (n=2200)
- occurring
- c levels of 66
- 10 µg/L.
- **ancy at 8 and 30**
- TAs was
- Looked for associations between measures at each of these time points and neurodevelopmental outcomes, unable to limit exposure to just prenatal or early life.

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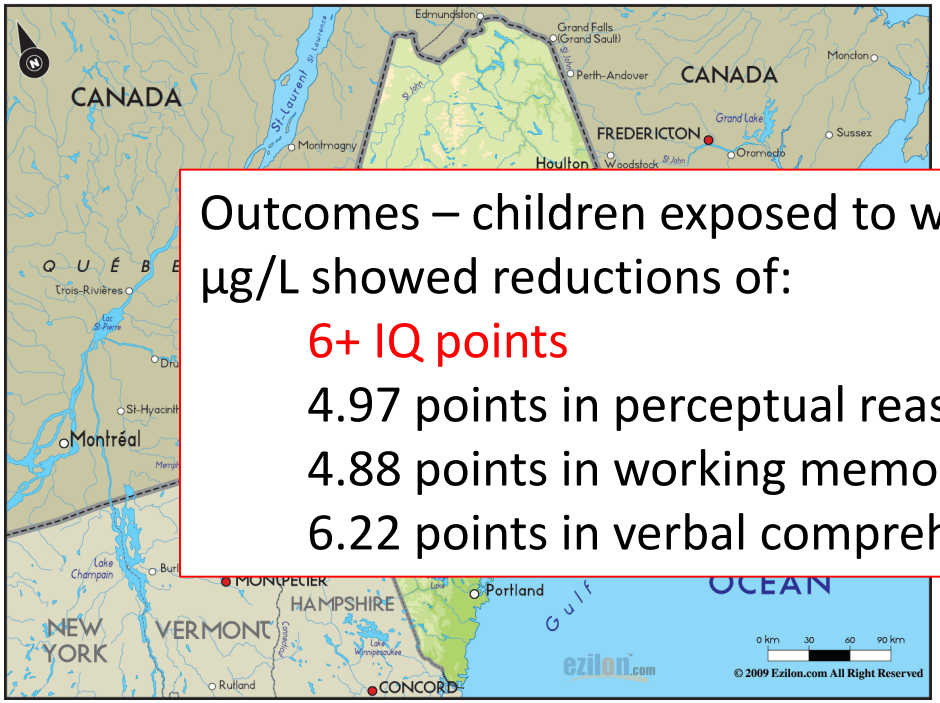
Prenatal and early life exposure to arsenic in Maine

- Contamination a result of naturally occurring inorganic arsenic
- Children (grades 3-5) in the school districts surrounding Augusta, Maine and use well water in their homes (n=272)
- Drinking water measured at the tap had average arsenic levels with range of 9.88 to 115.3 µg/L.
- 31.25% of households had arsenic measurements above 10 µg/L
- Possible in utero exposure in some children, but unable to distinguish this from childhood arsenic exposure.

Wasserman, G. A., Liu, X., Loiacono, N. J., Kline, J., Factor-Litvak, P., van Geen, A., . . . Graziano, J. H. (2014). A cross-sectional study of well water arsenic and child IQ in Maine schoolchildren. *Environ Health, 13*(1), 23. doi: 10.1186/1476-069x-13-23

Prenatal and early life exposure to arsenic in Maine



- Contamination a result of naturally occurring inorganic arsenic

l districts
use well water in

ap had average
o 115.3 µg/L.
c measurements

e children, but
ildhood arsenic
exposure.

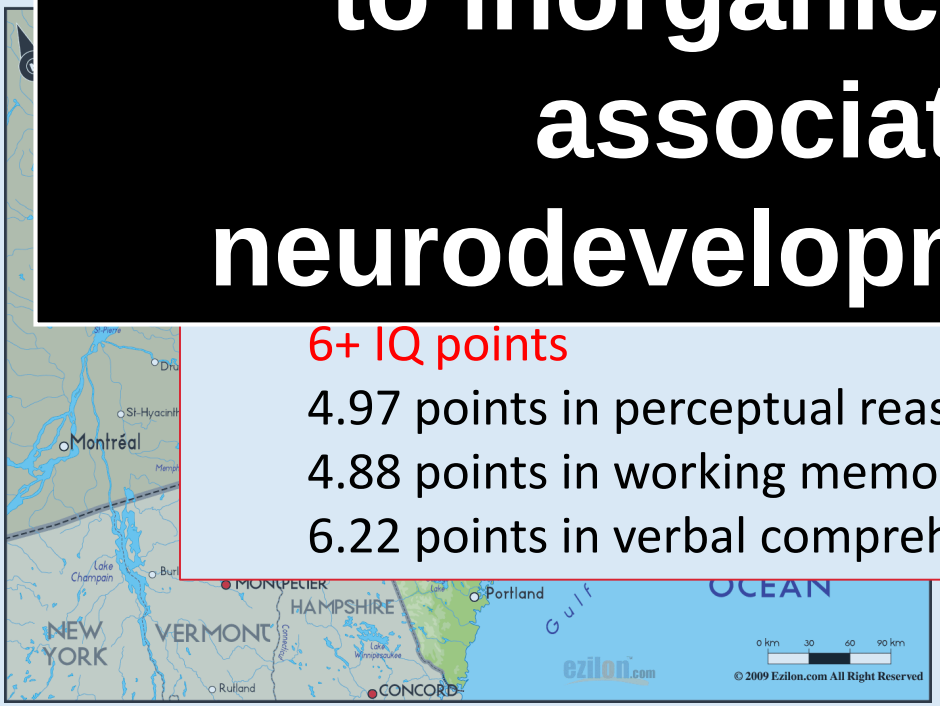
Outcomes – children exposed to well water arsenic levels ≥ 5 µg/L showed reductions of:

- 6+ IQ points
- 4.97 points in perceptual reasoning scores
- 4.88 points in working memory
- 6.22 points in verbal comprehension.

Wasserman, G. A., Liu, X., Loiacono, N. J., Kline, J., Factor-Litvak, P., van Geen, A., . . . Graziano, J. H. (2014). A cross-sectional study of well water arsenic and child IQ in Maine schoolchildren. *Environ Health, 13*(1), 23. doi: 10.1186/1476-069x-13-23

Prenatal and early life exposure to arsenic in Maine

Prenatal and early life exposures to inorganic arsenic are associated with neurodevelopmental deficits



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Prenatal and early life exposure to arsenic in Chile

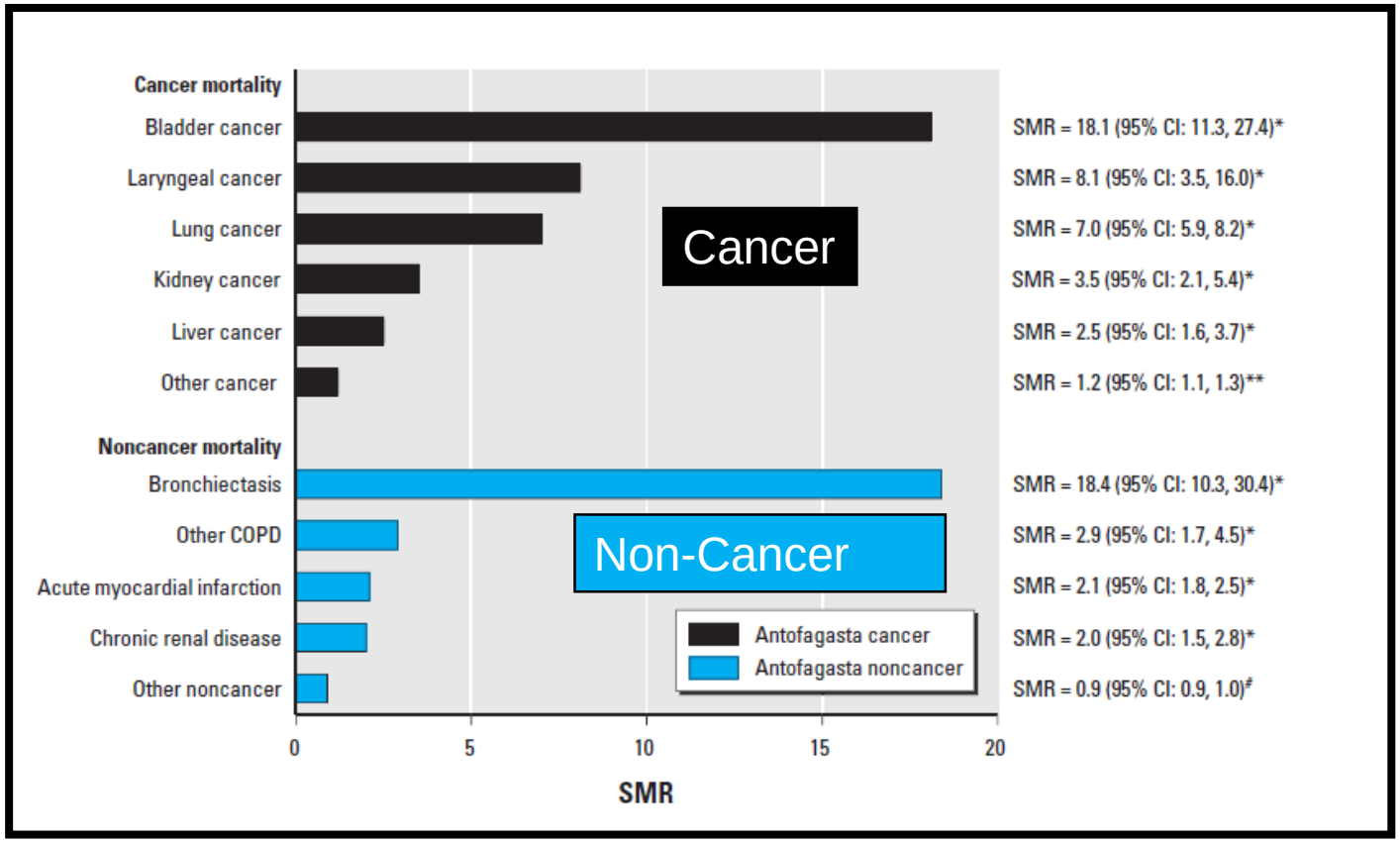
- Prior to 1958, was 90 ppb, 1958-1970 was 870 ppb, 1970 went to 110 ppb

- Birth Cohort 1: Children in Antofagasta and Mejillones, Chile, born during 1958-1970
- Water: estimated drinking water to have >800 µg/L iAs when born have probable in utero exposure to arsenic (but also probable early life exposure as well)

- Birth Cohort 2: Children in Antofogasta and Mejilones, Chile, Born during 1940-1957, Have probable childhood exposure during the high arsenic period, but not in utero exposure

Smith, A. H., Marshall, G., Liaw, J., Yuan, Y., Ferreccio, C., & Steinmaus, C. (2012). Mortality in Young Adults following in Utero and Childhood Exposure to Arsenic in Drinking Water. *Environmental health perspectives, 120*(11), 1527-1531. doi: 10.1289/ehp.1104867

Prenatal and early life exposure to arsenic is associated with **later life** disease



Studies suggest potential developmental reprogramming associated with later life disease

Smith et al 2012, Liaw et al., 2008; Smith et al., 2006

Morinaga Milk: Mass arsenic poisoning in Japan

Okayama prefecture in Western Japan

- Japanese infants given formula milk from the Morinaga company in 1955
- Between 12,000-14,000 people estimated/reported
- Contaminated dry milk formula with an estimated 4-7 mg/L arsenic.
- Estimated daily exposure to be >500 µg/kg body weight

- Infancy/Early life exposure window isolated

- Cohort now 50+ years old and have:
- Increased **liver, pancreas, skin, and hematopoietic cancers c**ompared with siblings born after exposure

Dakeishi, M., Murata, K., & Grandjean, P. (2006). Long-term consequences of arsenic poisoning during infancy due to contaminated milk powder. *Environ Health, 5*, 31. doi: 10.1186/1476-069x-5-31

Morinaga Milk: Mass arsenic poisoning in Japan

Prenatal and early life exposures to inorganic arsenic are associated with cancer later in life

- Contaminated dry milk formula with an estimated 4-7 mg/L arsenic.
- Estimated daily exposure to be >500 µg/kg body weight
- Infancy/Early life exposure window isolated
- Cohort now 50+ years old and have:
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Prenatal and early life exposure to arsenic is associated with **early** and **later life** diseases

Observations:

1) Poorer birth outcomes
2) Increased risk for infection
3) Neurodevelopmental effects
4) Cancer

Knowledge Gaps: The Need for Animal Model Research

"Detailed molecular analyses in animal models that employ gene knockouts or chemical inhibition of targeted pathways are required to elucidate the precise mechanisms linking iAs exposure and adverse health effects."

Environ Health Perspect; DOI:10.1289/ehp.1409360

Mechanisms Underlying Latent Disease Risk Associated with Early-Life Arsenic Exposure: Current Research Trends and Scientific Gaps

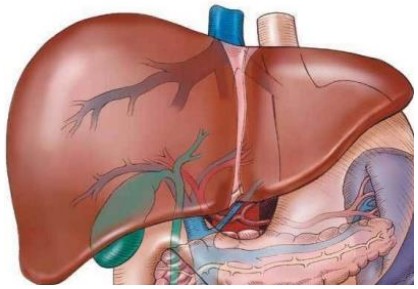
Kathryn A. Bailey[1], Allan H. Smith[2], Erik J. Tokar[3], Joseph H. Graziano[4], Kyoung-Woong Kim[5], Panida Navasumrit[6], Mathuros Ruchirawat[6], Apinya Thiantanawat[6], William A. Suk[7], and Rebecca C. Fry[1]

What are the relationships among levels of inorganic arsenic, timing of exposure, and health outcomes in **mice**?

Prenatal and early life exposure to arsenic is associated with **later life** disease

CD1 mice/85 ppm iAs

increase in hepatocellular carcinomas



DNA methylation
Stable gene expression

Prenatal exposure → pup → adulthood

Prenatal exposure to arsenic is associated with **later life** disease

- C3H mice, drinking water with sodium arsenite at 0, 42.5, and 85 ppm, Prenatal exposure: **days 8-18 of gestation**

- Outcomes –
  - **males**: increased hepatocellular carcinomas and adrenal tumors
  - **females:** increased hyperplasia of uterus and oviduct, phorbol ester exposure used as a promotor - promoted lung tumors in both, liver tumors in females
  - **Epigenetic mechanisms:** A loss of DNA methylation in GC-rich regions in the newborn mouse liver and aberrant expression of glutathione utilization system, insulin-like growth factor signaling, and stress response related genes.

"Induction of tumors of the liver, lung, ovary and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers" – Carcinogenesis 2004. Waalkes et al.

"Aberrant DNA methylation and gene expression in livers of newborn mice transplacentally exposed to a hepatocarcinogenic dose of inorganic arsenic" Toxicology (2007) – Xie et al.

Prenatal exposure to arsenic is associated with **later life** disease

- CD1 mice, 12.5 or 25 ppm MMA3+ in drinking water, Prenatal exposure: days 8-18 of gestation

- Outcomes – sex dependent
  - **females:** epithelial uterine tumors, oviduct hyperplasia, adrenal cortical adenoma, ovarian tumors
  - **males:** hepatocellular carcinoma, adrenal adenoma, lung adenocarcinoma.

"Tumors and Proliferative Lesions in Adult Offspring After Maternal Exposure to Methylarsonous Acid During Gestation in CD1 Mice" – Tokar et al., 2012. Archives of Toxicology

Whole life exposure to arsenic is associated with **later life** disease

- CD1 mice, drinking water with sodium arsenite at 0, 6, 12, or 24 ppm or 0, 50, 500, 5000 ppb sodium arsenite in drinking water "whole life" = prenatal, childhood, and adulthood.

- **Outcomes 1 (ppm)**– adenocarcinoma, hepatocellular carcinoma, gallbladder tumors (males) and uterine carcinomas, adrenal tumors, ovarian tumors.

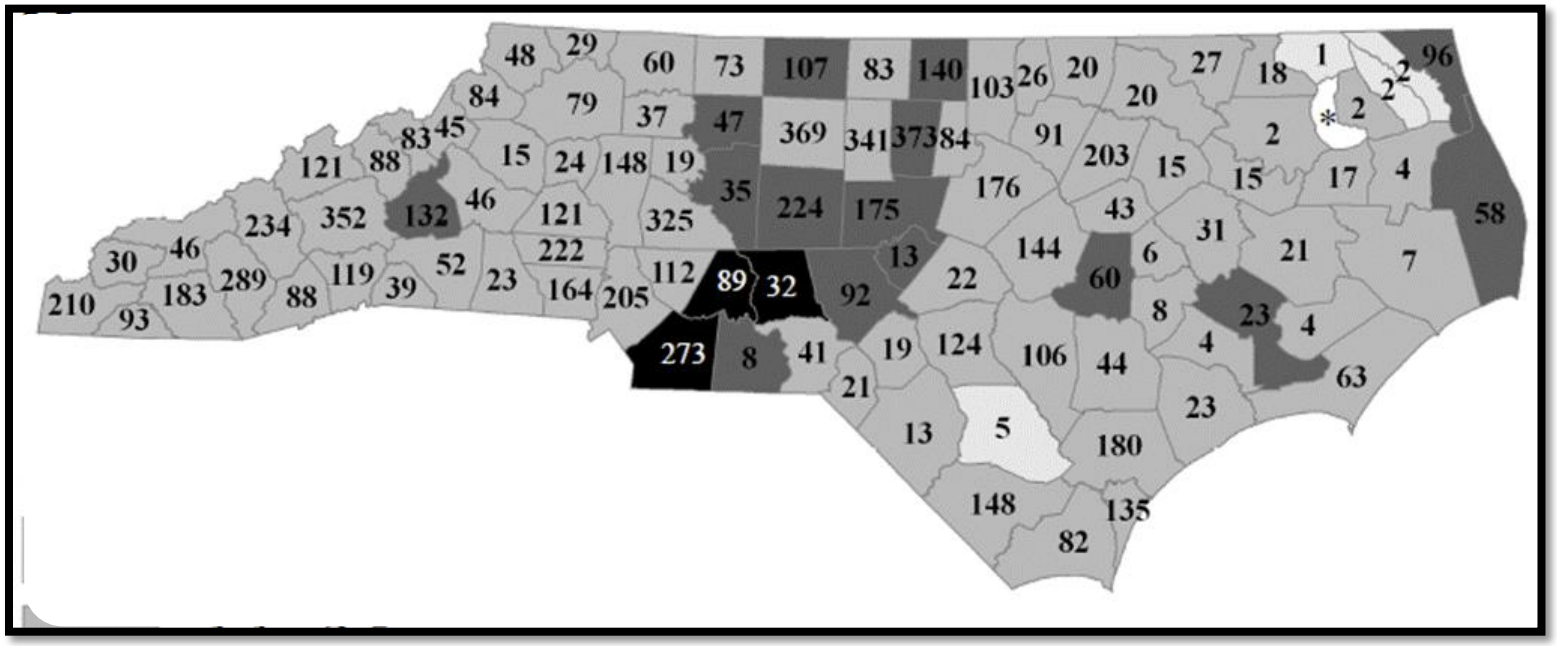
- **Outcomes 2 (ppb)**-
  - **males:** increased bronchio-alveolar tumor incidence at 50, 500, and 5000 ppb.
  - **females:** increased lung adenoma in 50 ppb dose

"Carcinogenesis Effects of "Whole-Life" Exposure to Inorganic Arsenic in CD1 mice" – Tokar et al, 2011. Toxicological Sciences

Lung tumors in mice induced by "whole-life" inorganic arsenic exposure at human-relevant doses". Archives of Toxicology (2014). Waalkes et al.

Are their local impacts of inorganic arsenic exposure?

Arsenic and other toxic metals are contaminating the water of North Carolinians



Sanders et al. Environ Int 2012

>63,000 wells over 10 yrs
1436 wells >10 ppb
Hundreds > 50 ppb
Max=800 ppb
More than 2 million are on private well water

Arsenic and other toxic metals are contaminating the water of North Carolinians

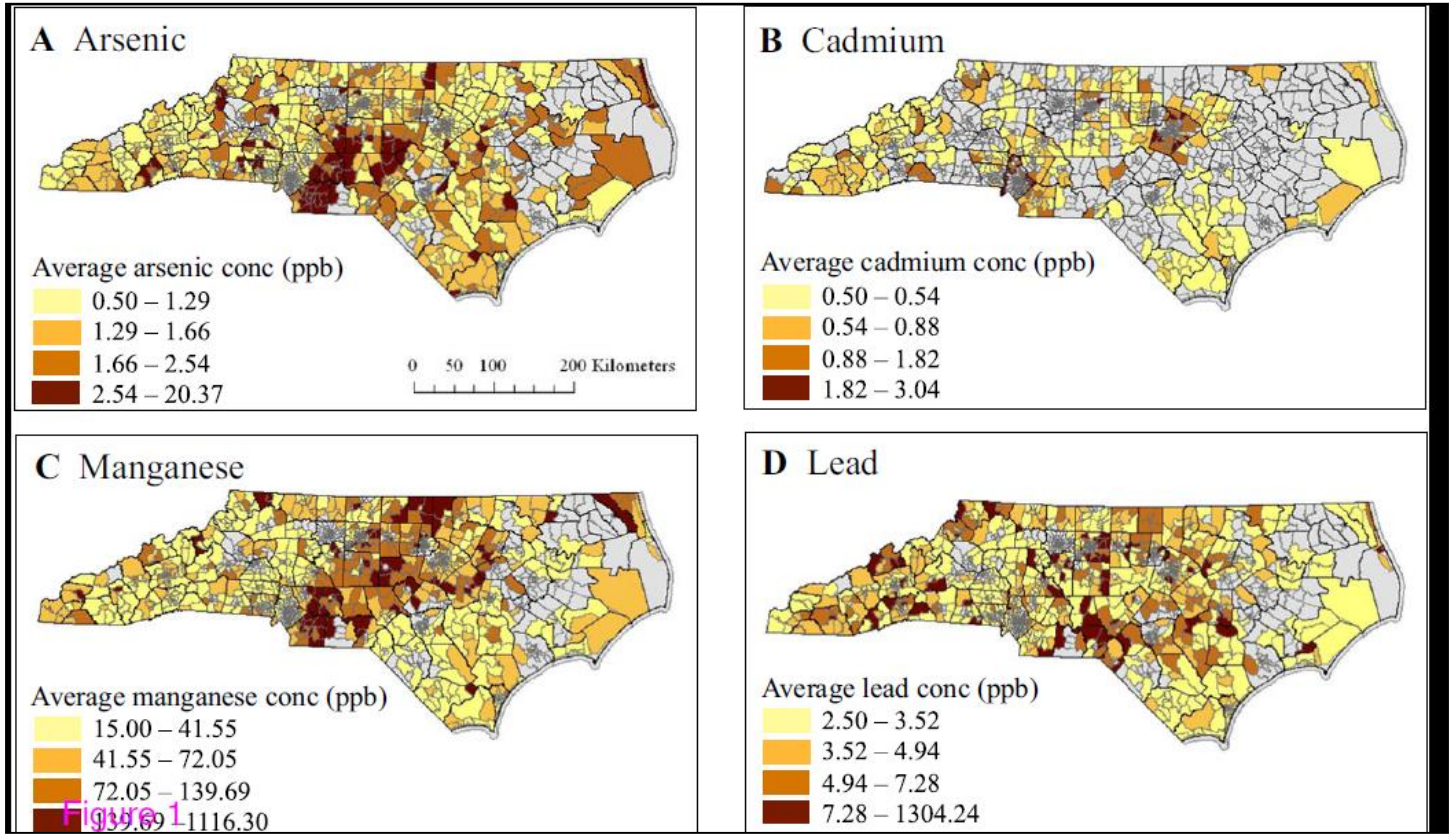


Figure 1

Sanders et al. BMC Public Health 2014

- Arsenic and manganese co-occur
- North Carolina Birth Defects Monitoring Program
- Increased prevalence of birth defects in counties where toxic metals are high, mn with conotruncal defects

Sanders *et al. BMC Public Health* 2014, **14**:955
http://www.biomedcentral.com/1471-2458/14/955

BMC Public Health

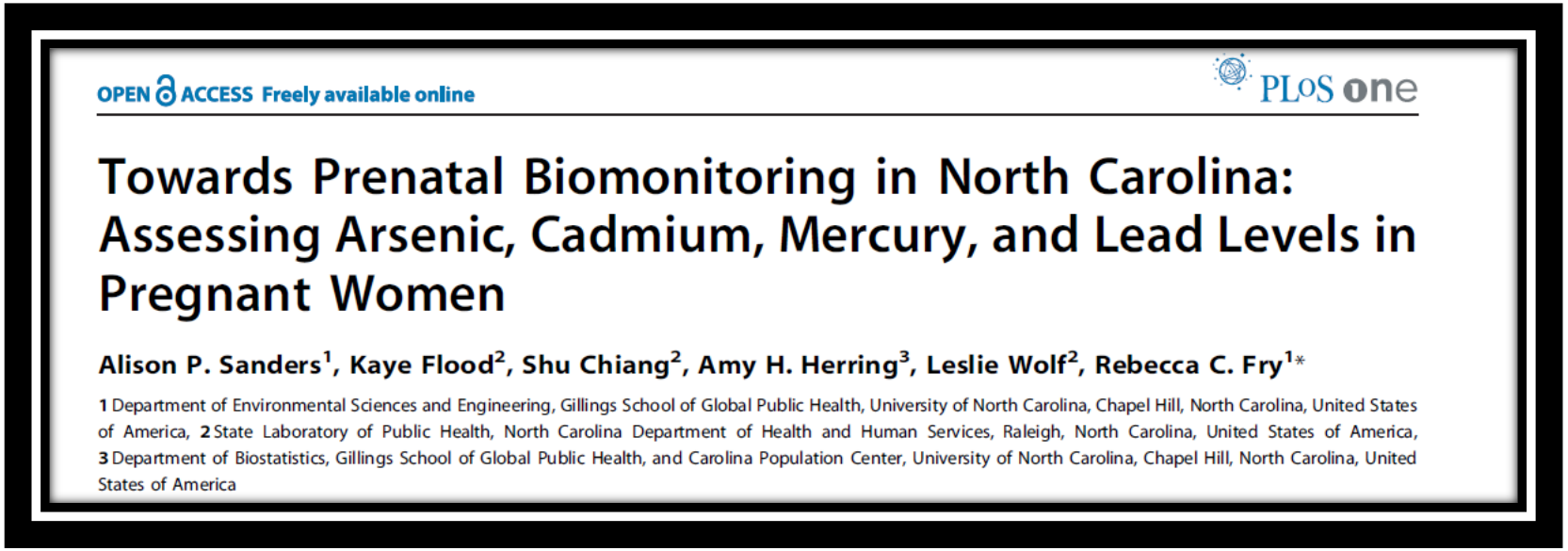
RESEARCH ARTICLE Open Access

Association between arsenic, cadmium, manganese, and lead levels in private wells and birth defects prevalence in North Carolina: a semi-ecologic study

Alison P Sanders[1,7], Tania A Desrosiers[2,6], Joshua L Warren[3,8], Amy H Herring[3], Dianne Enright[4], Andrew F Olshan[2], Robert E Meyer[5,6] and Rebecca C Fry[1*]

In counties in NC where toxic metals are high, there is increased prevalence of specific birth defects

Public health considerations



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PLoS one

Towards Prenatal Biomonitoring in North Carolina: Assessing Arsenic, Cadmium, Mercury, and Lead Levels in Pregnant Women

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- **Questions related to water sources for drinking in the home? Water testing? Prenatal biomonitoring?**

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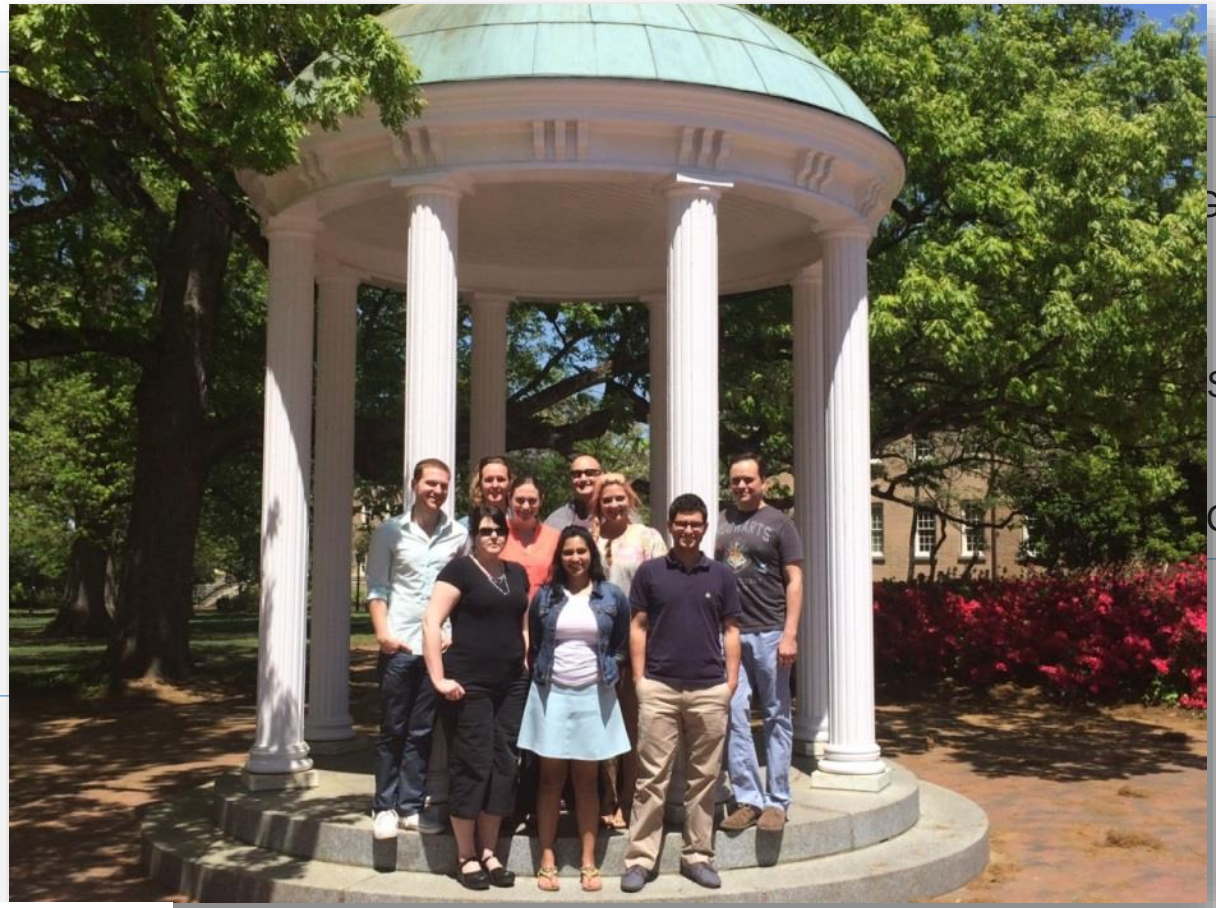

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