



Current Understanding of Mechanisms Underlying Arsenic-Induced Developmental Toxicity

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Conflict of Interest Statement

- I have no conflicts of interest to report.



Objectives

1. Describe the health impacts of *in utero* exposure to arsenic on early and later-in-life health
2. Discuss epigenetic mechanisms that may link such developmental origins of health and disease
3. Highlight methods to assess dose-response of epigenetic effects
4. Highlight sexual dimorphism in arsenic-induced health effects



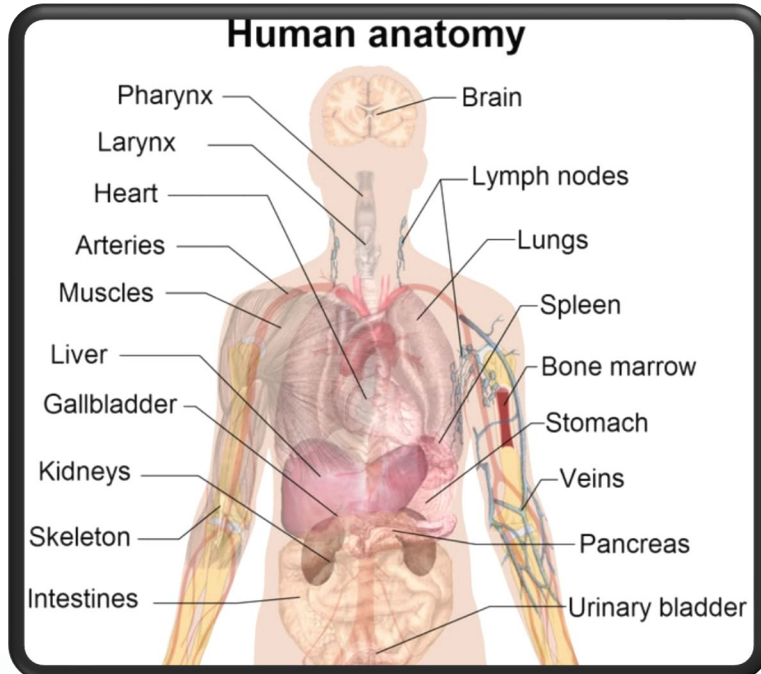
Inorganic Arsenic Continues to Contaminate the Water of Millions Around the Globe



Hundreds of millions of individuals are exposure to high levels of arsenic in drinking water



Arsenic Is Associated with Both Cancer and Non-Cancer Endpoints



- Classified as Group 1 Carcinogen by the International Agency for Research on Cancer (IARC)
- Chronic exposure results in many cancers: **skin, bladder, lung, liver, prostate and kidney**
- Exposure is associated with non-cancer endpoints: neurological disorders, reproductive effects, cardiovascular disease, diabetes



Early Life Health Effects of Inorganic Arsenic

- Birthweight (growth restriction)
- Immune dysfunction-increased risk for infections
- Increased mortality
- Cognitive impairments in children



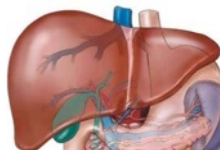
Early Life Exposures Associated with Later in Life Health Effects

Mice

CD1 mice



hepatocellular carcinomas



Prenatal exposure

adulthood

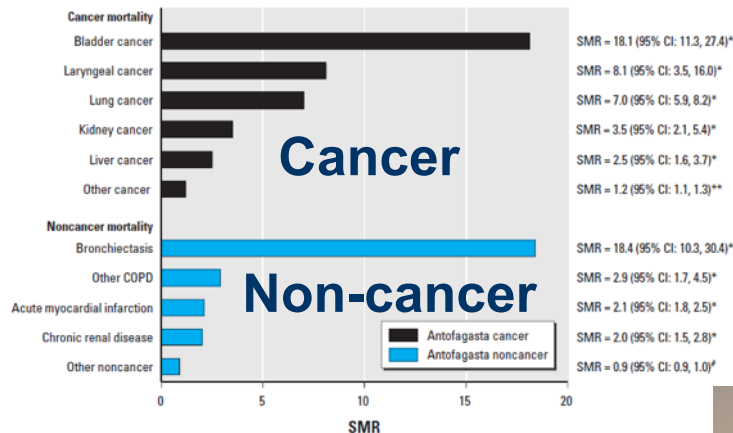
Permanent changes in gene expression

Waalkes, M. et al. 2004. Xie, Y. et al. 2007.



M Waalkes

Humans



Smith et al. 2012



A Smith



Early Life Exposures Associated with Later in Life Health Effects

Mice

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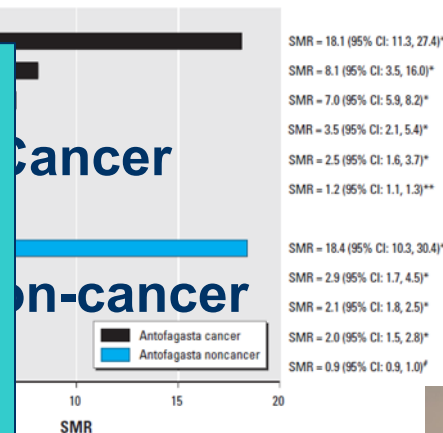
hepatocellular

Prenatal exposure

Permanent changes

Waalkes, M. et al.

Arsenic is a model contaminant for the study of the developmental origins of health and disease hypothesis DoHAD



Smith et al. 2012



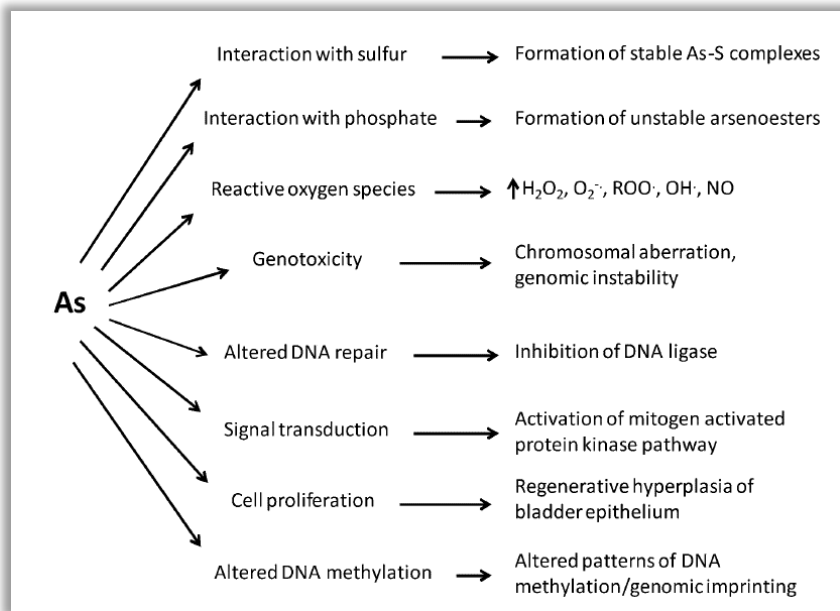
M Waalkes



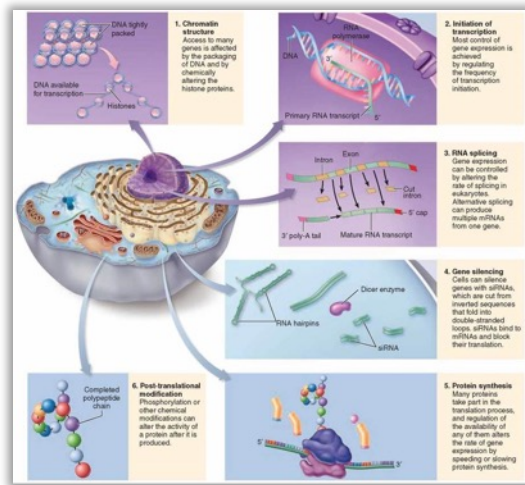
A Smith



Early Life Exposures Associated with Later in Life Health Effects



Cellular and molecular machinery



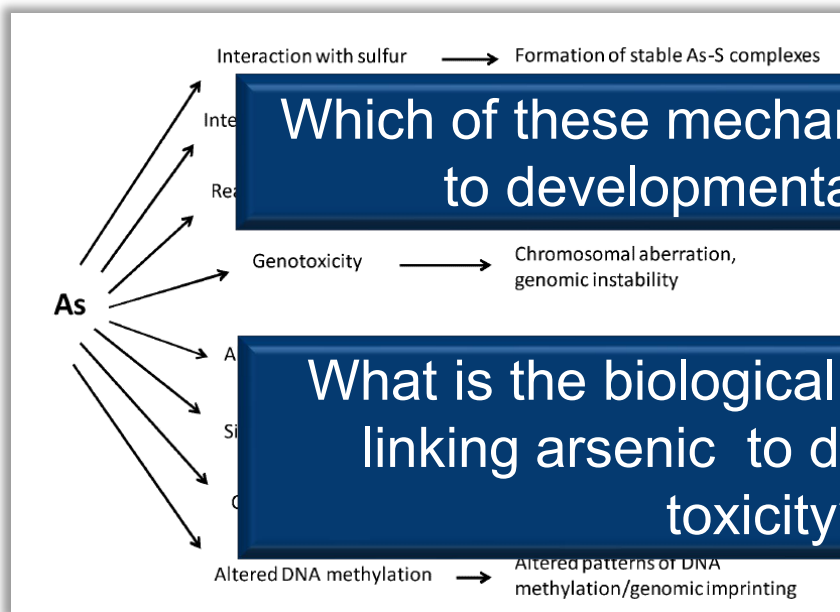
Biomarkers:

Epigenetic, Genomic, Proteomic, Metabolomic, Inflammatory responses,

Michael Hughes, et al Tox Sci 132(2) 305-332 (2011)



Early Life Exposures Associated with Later in Life Health Effects



Which of these mechanisms is relevant to developmental toxicity?

What is the biological chain of events linking arsenic to developmental toxicity?

Cellular and molecular machinery

- 1. Chromatin structure**
- 2. Initiation of transcription**
Most control of gene expression is achieved by regulating the frequency of transcription initiation.
- 3. RNA splicing**
Gene expression can be controlled by altering the rate of splicing in eukaryotes. Alternative splicing can produce multiple mRNAs from one gene.
- 4. Gene silencing**
Cells can silence genes with siRNAs, which are cut from inverted sequences that fold into double-stranded loops. siRNAs bind to mRNAs and block their translation.
- 5. Protein synthesis**
Many proteins take part in the translation process, and regulation of any of them alters the rate of gene expression by speeding or slowing protein synthesis.

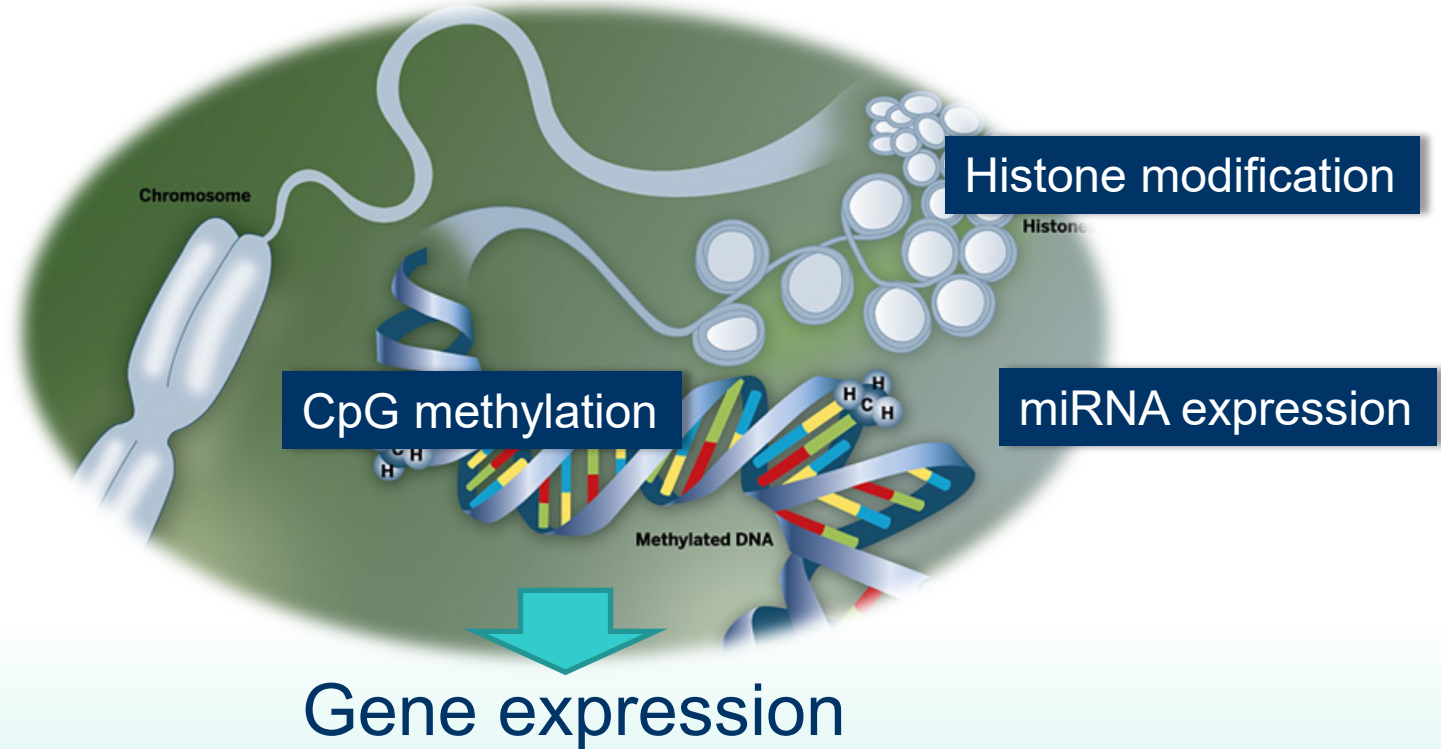
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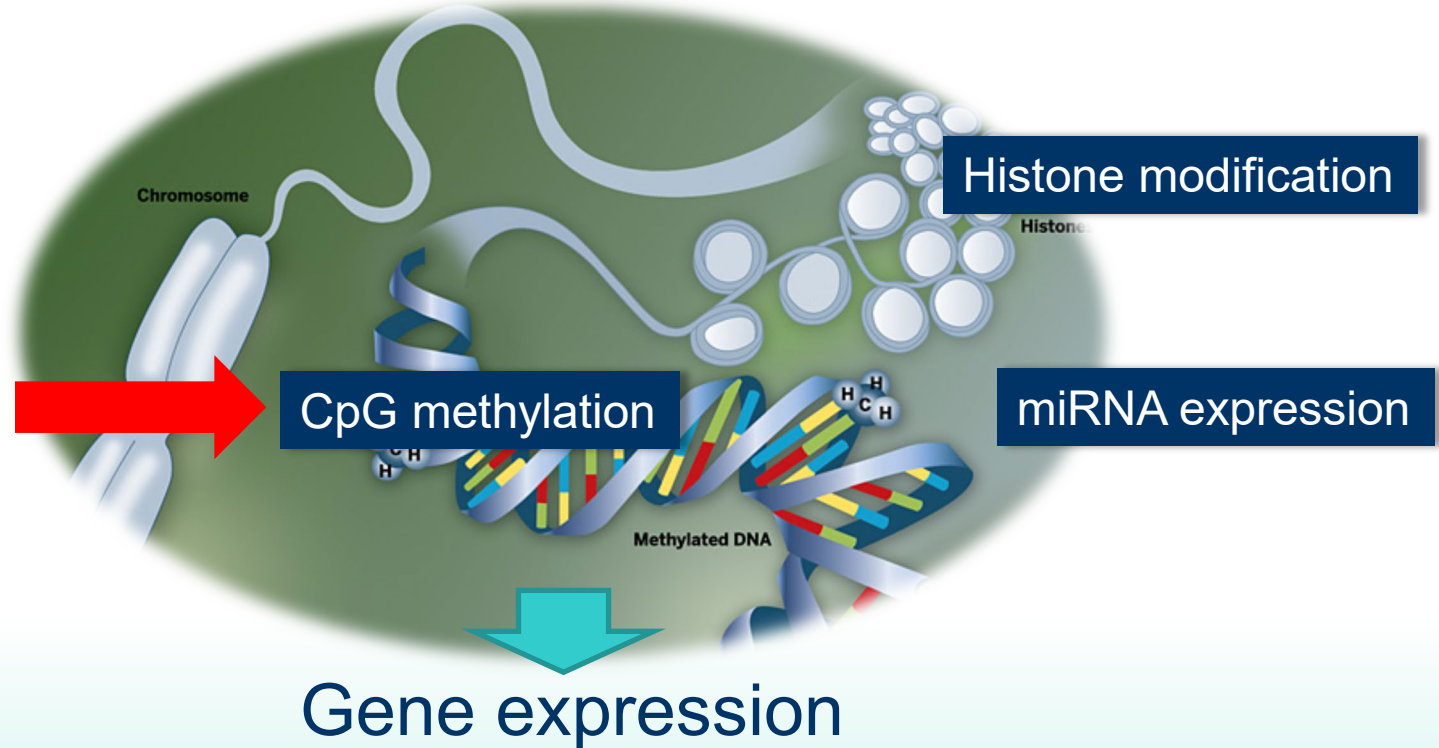
Michael Hughes, et al Tox Sci 132(2) 305-332 (2011)



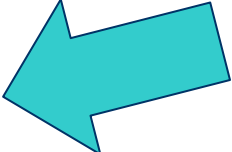
Do Epigenetic Mechanisms Underlie Health Effects Associated with Early Life Exposure?



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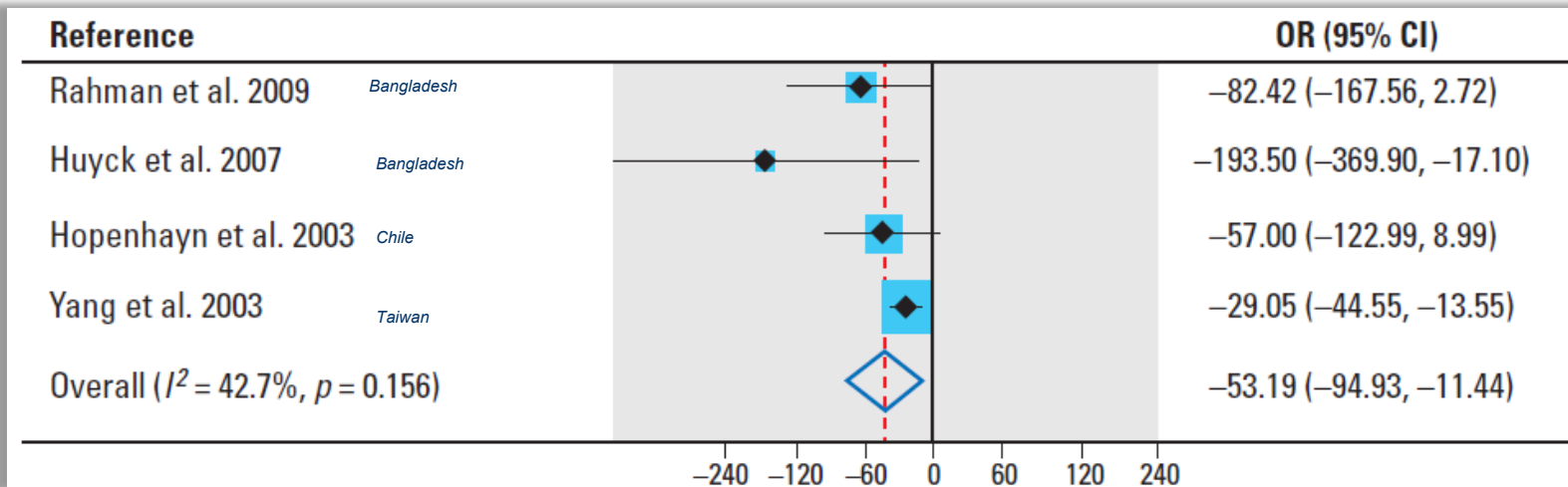


Early Life Health Effects of Inorganic Arsenic

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- Increased mortality
- Cognitive impairments in children



Prenatal Arsenic Exposure is Associated with Detrimental Birth Outcomes: Lower Birthweight



Mexico. Laine et al. *EHP*. 2015 Feb; 123 (2); Arsenic range: 0-236 ppb

Bangladesh. Kile et al. *Epidemiology*. 2016 Mar; 27(2): 173–181; Arsenic range: 0-1400 ppb

United States, Oklahoma. Henn et al. *EHP*. 2016 Aug; 124 (8); Maternal and umbilical cord blood arsenic, 1.0–2.3 and 1.8–3.3 $\mu\text{g/L}$



Prenatal Arsenic Exposure is Associated with Detrimental Birth Outcomes: Lower Birthweight

Reference

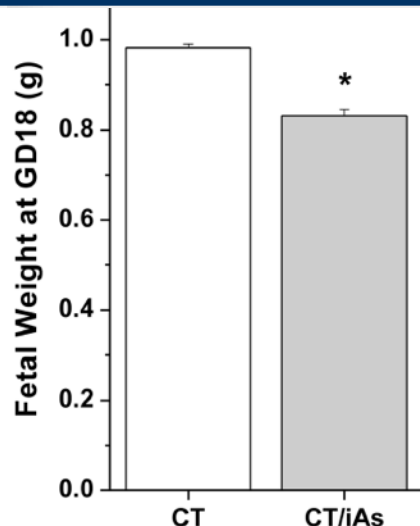
Rahman et al. 20

Huyck et al. 2007

Hopenhayn et al

Yang et al. 2003

Overall ($I^2 = 42.7\%$)



95% CI)

167.56, 2.72)

369.90, -17.10)

122.99, 8.99)

44.55, -13.55)

94.93, -11.44)

The Epigenetic Effects of a High Prenatal Folate Intake in Male Mouse Fetuses Exposed *In Utero* to Arsenic

Verne Tsang^a, Rebecca C. Fry^b, Mihai D. Niculescu^c, Julia E. Rager^b, Jesse Saunders^a, David S. Paul^a, Steven H. Zeisel^{a,c}, Michael P. Waalkes^d, Miroslav Stýblo^a, and Zuzana Drobná^{a,*}



Elevated Levels of Inorganic Arsenic in Water in Mexico



Research | Children's Health

A Section 508-compliant HTML version of this article is available at <http://dx.doi.org/10.1289/ehp.1307476>

Maternal Arsenic Exposure, Arsenic Methylation Efficiency, and Birth Outcomes in the Biomarkers of Exposure to Arsenic (BEAR) Pregnancy Cohort in Mexico

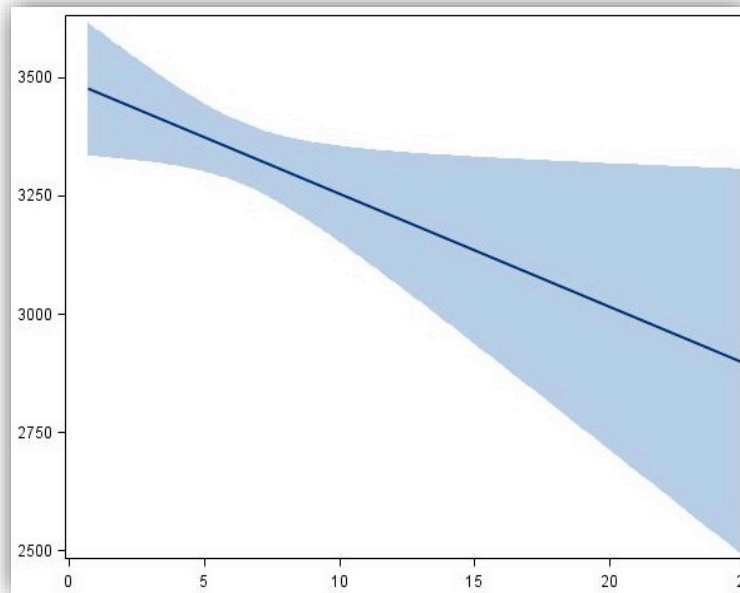
Jessica E. Laine,¹ Kathryn A. Bailey,² Marisela Rubio-Andrade,³ Andrew F. Olshan,^{1,4} Lisa Smeester,² Zuzana Drobná,⁵ Amy H. Herring,^{1,6} Miroslav Styblo,^{7,8} Gonzalo G. Garcia-Vargas,⁹ and Rebecca C. Fry⁷

¹Department of Epidemiology, and ²Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Gillings School of Public Health, Chapel Hill, North Carolina, USA; ³Facultad de Medicina, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, Mexico; ⁴Carolina Population Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; ⁵Department of Nutrition, and ⁶Department of Biostatistics, University of North Carolina at Chapel Hill, Gillings School of Public Health, Chapel Hill, North Carolina, USA



Laine et al, 2014 EHP

Birth weight (grams)

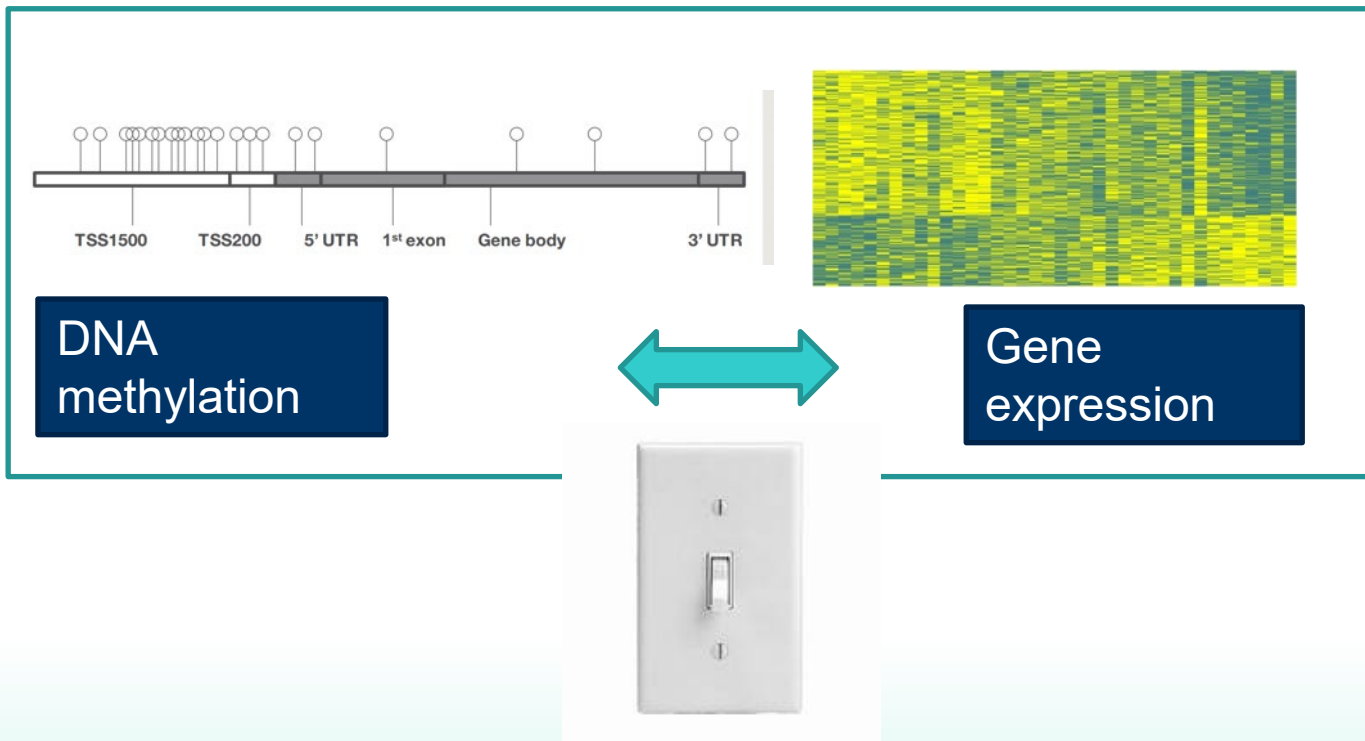


Arsenic exposure-%MMAs

G Garcia-Vargas

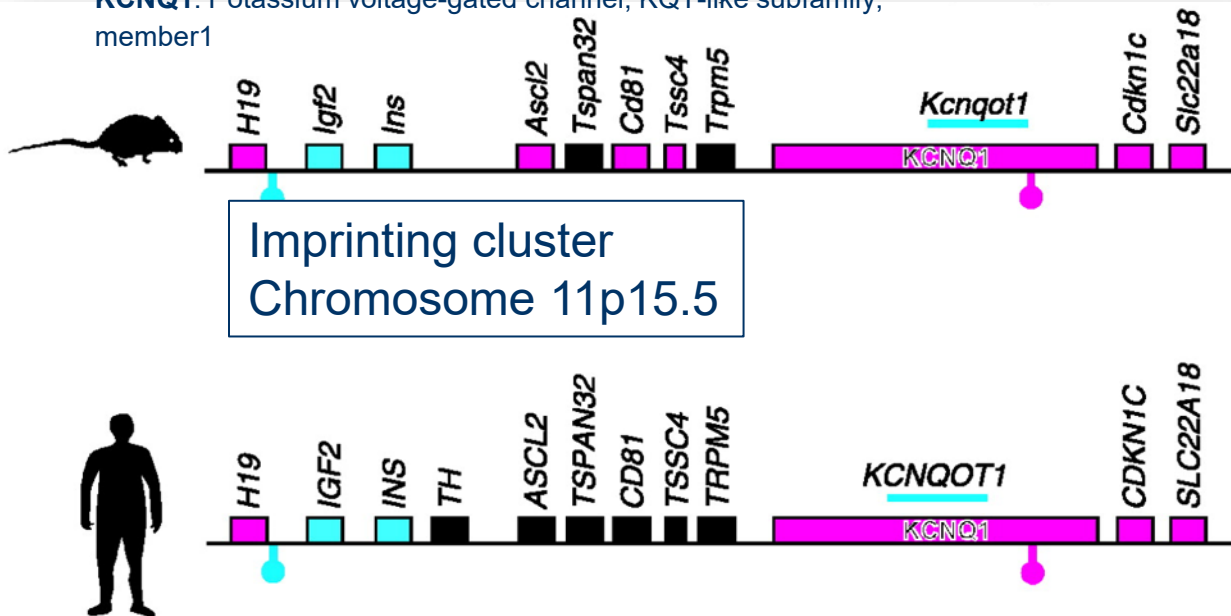
SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

Impact of CpG Methylation on Gene Expression Assessed Genome-wide Using the Illumina 450k Assay



The Imprinted Gene KCNQ1 has Altered CpG Methylation in Relationship to Prenatal Arsenic Exposure

KCNQ1: Potassium voltage-gated channel, KQT-like subfamily, member1



Blue indicates paternally expressed alleles

Pink indicates maternally expressed alleles

Black indicates non-imprinted genes and

Grey indicates those not yet investigated

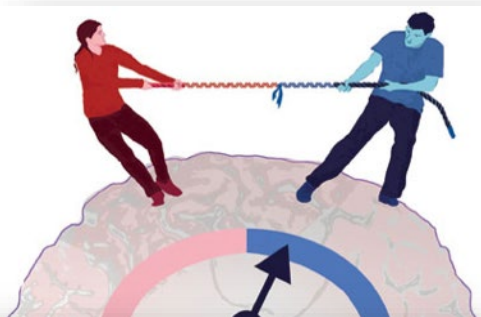
Periconceptual exposure to famine during the Dutch Hunger Winter (1944-45) linked to decreased methylation of Insulin-like growth factor 2 (Heijmans, PNAS 2008)

KCNQ1 differential methylation has been associated with gestational age. Lee et al 2012, Kwak et al Horm Res Paediatr 2010;74:333-33

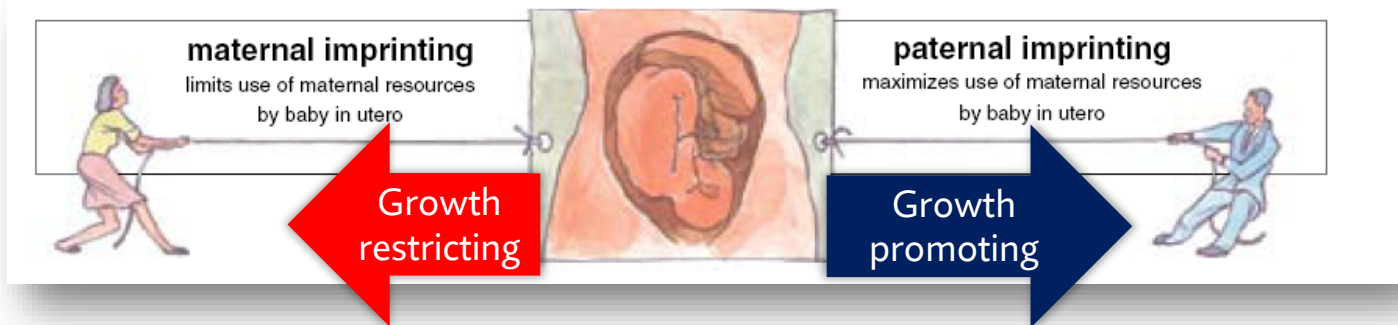


Imprinted Genes Are Tied to Size at Birth

Imprinted genes defy rules of Mendelian genetics with their expression tied to the parent from whom each allele was inherited.



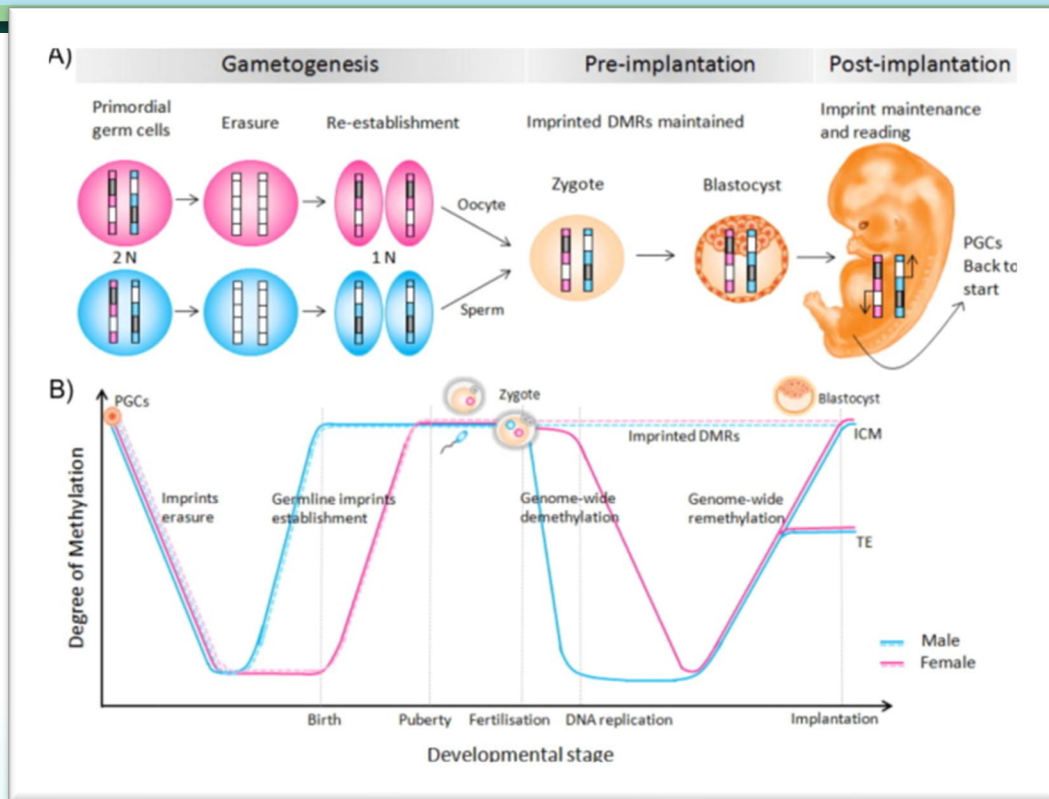
Genomic tug-of-war between mothers and fathers over the use of maternal resources by the fetus.



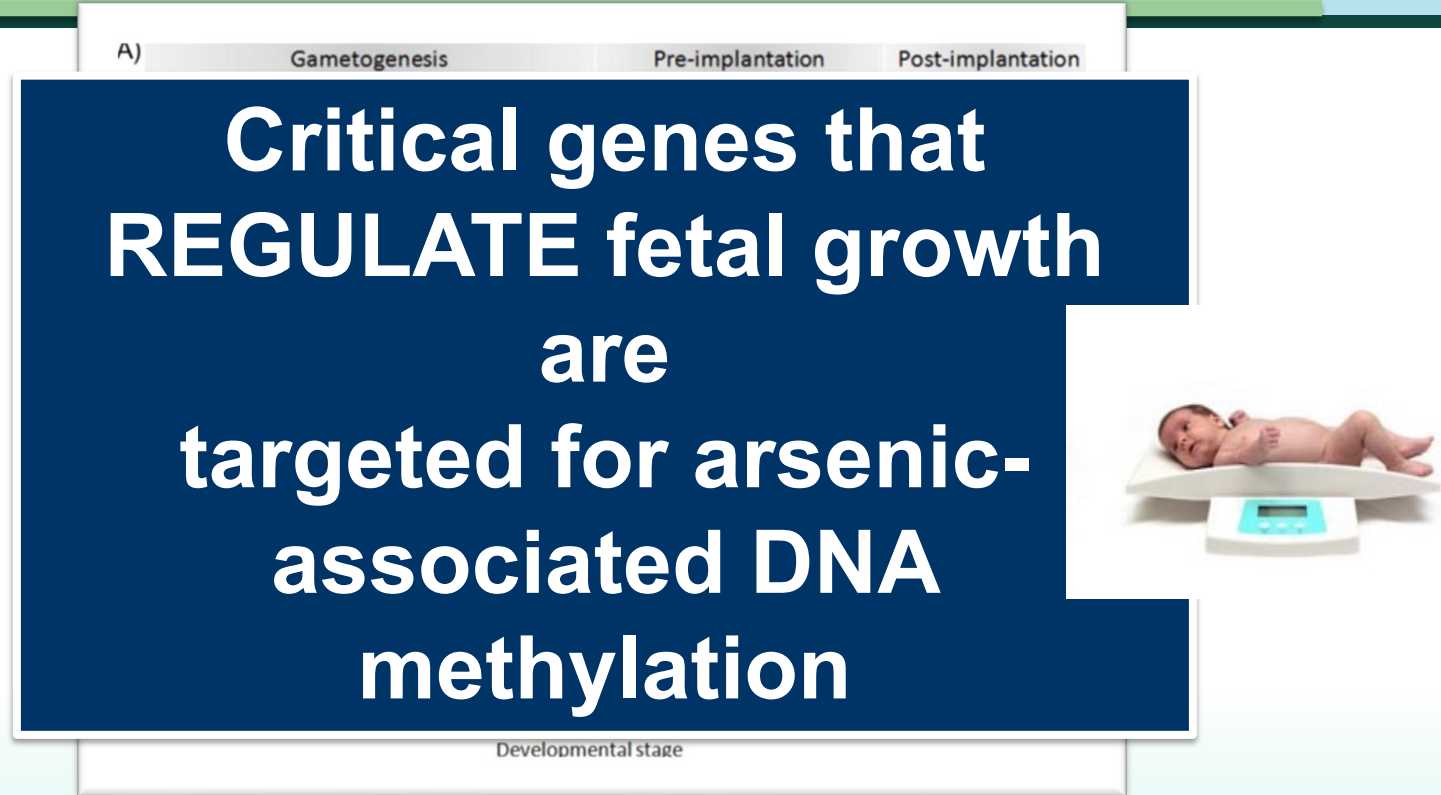
Jirtle and Weidman, American Scientist, March 2007



Imprinted Genes Are Tied to Size at Birth



Imprinted Genes Are Tied to Size at Birth



Imprinted Genes-a Link Between Preconception Arsenic and Health Outcomes

[Chem Res Toxicol](#). Author manuscript; available in PMC 2020 Jan 3.

PMCID: PMC6941420

Published in final edited form as:

NIHMSID: NIHMS1063417

[Chem Res Toxicol](#). 2019 Aug 19; 32(8): 1487–1490.

PMID: [31251040](#)

Published online 2019 Jul 8. doi: [10.1021/acs.chemrestox.9b00107](#)

Effects of Preconception and in Utero Inorganic Arsenic Exposure on the Metabolic Phenotype of Genetically Diverse Collaborative Cross Mice

[Rebecca C. Fry](#),^{††¶} [Kezia A. Addo](#),^{††¶} [Timothy A. Bell](#),[§] [Christelle Douillet](#),⁺ [Elizabeth Martin](#),[‡] [Miroslav Styblo](#),^{††+} and [Fernando Pardo-Manuel de Villena](#)[§]

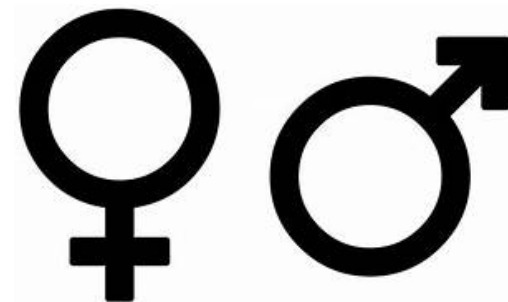
> [Arch Toxicol](#). 2020 Nov 3. doi: 10.1007/s00204-020-02941-w. Online ahead of print.

Sex-dependent effects of preconception exposure to arsenite on gene transcription in parental germ cells and on transcriptomic profiles and diabetic phenotype of offspring

[Abhishek Venkatratnam](#)^{1 2}, [Christelle Douillet](#)¹, [Brent C Topping](#)², [Qing Shi](#)¹, [Kezia A Addo](#)², [Folami Y Ideraabdullah](#)^{1 3}, [Rebecca C Fry](#)⁴, [Miroslav Styblo](#)⁵

Affiliations + expand

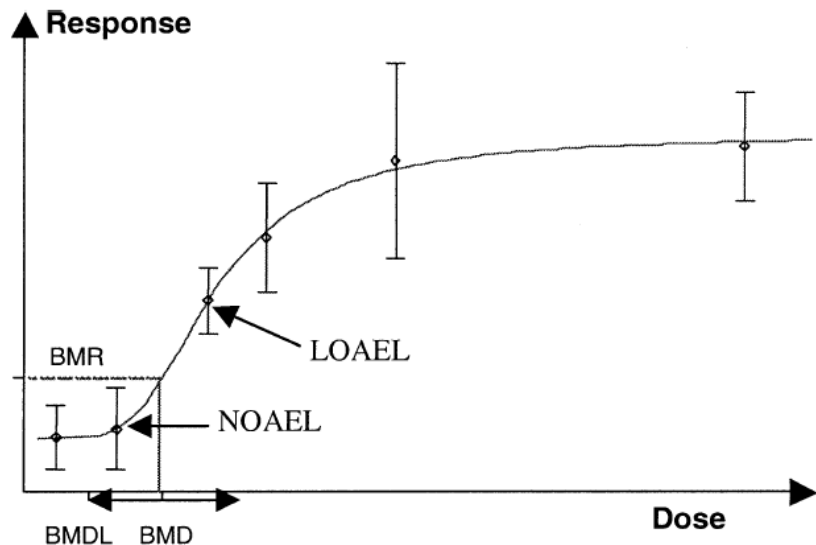
PMID: 33145626 DOI: [10.1007/s00204-020-02941-w](#)



M. Styblo



Applying a Benchmark Dose Approach to Epigenetic Datasets



- Mathematically model the relationship between quantified exposures (doses) and the change in the incidence or severity of a response
- Use various curve fits to model dose-response relationships
- Identify values to use in risk assessment calculations from the best fitting model curve

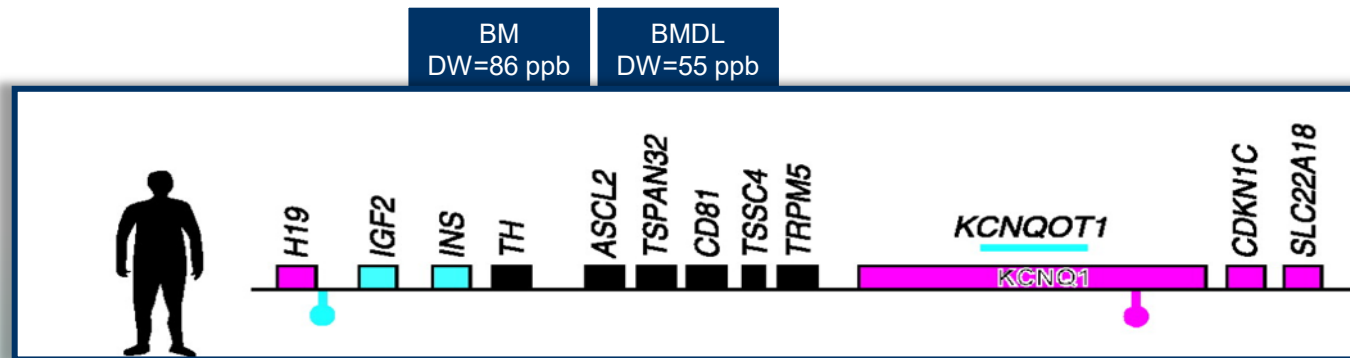
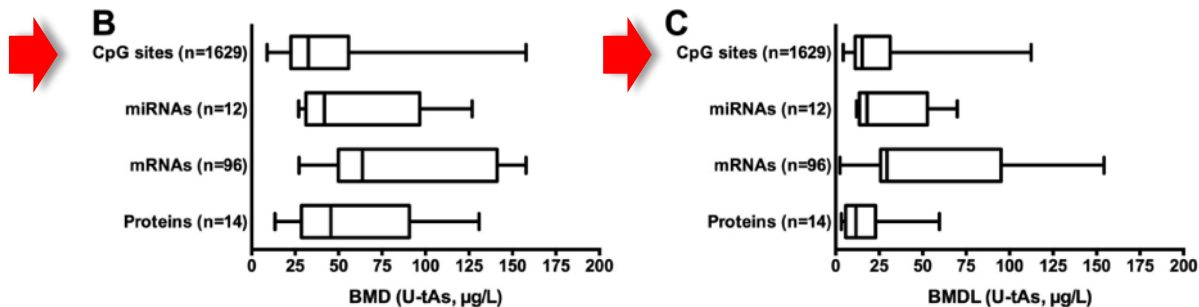


Julia Rager

LOAEL: Low Observed Adverse Effect Level
NOAEL: No Observed Adverse Effect Level
BMD: Benchmark Dose
BMDL: Benchmark Dose Lower Bound



Applying a Benchmark Dose Approach to Epigenetic Datasets



Applying a Benchmark Dose Approach to Epigenetic Datasets

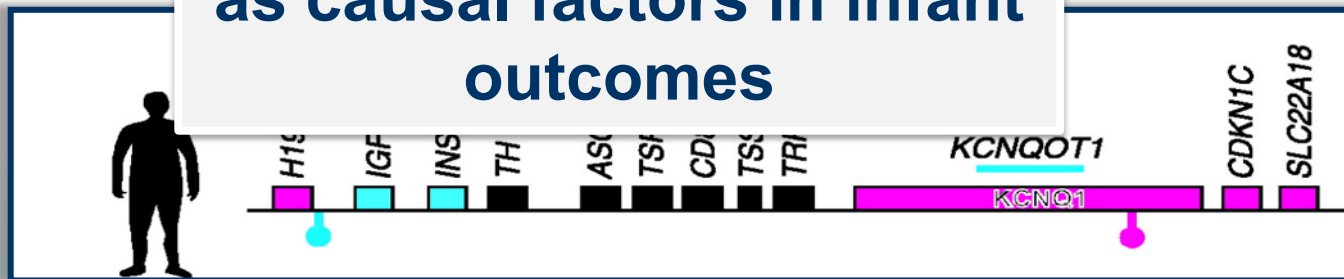


B
CpG sites (n
miRNAs
mRNAs
Proteins

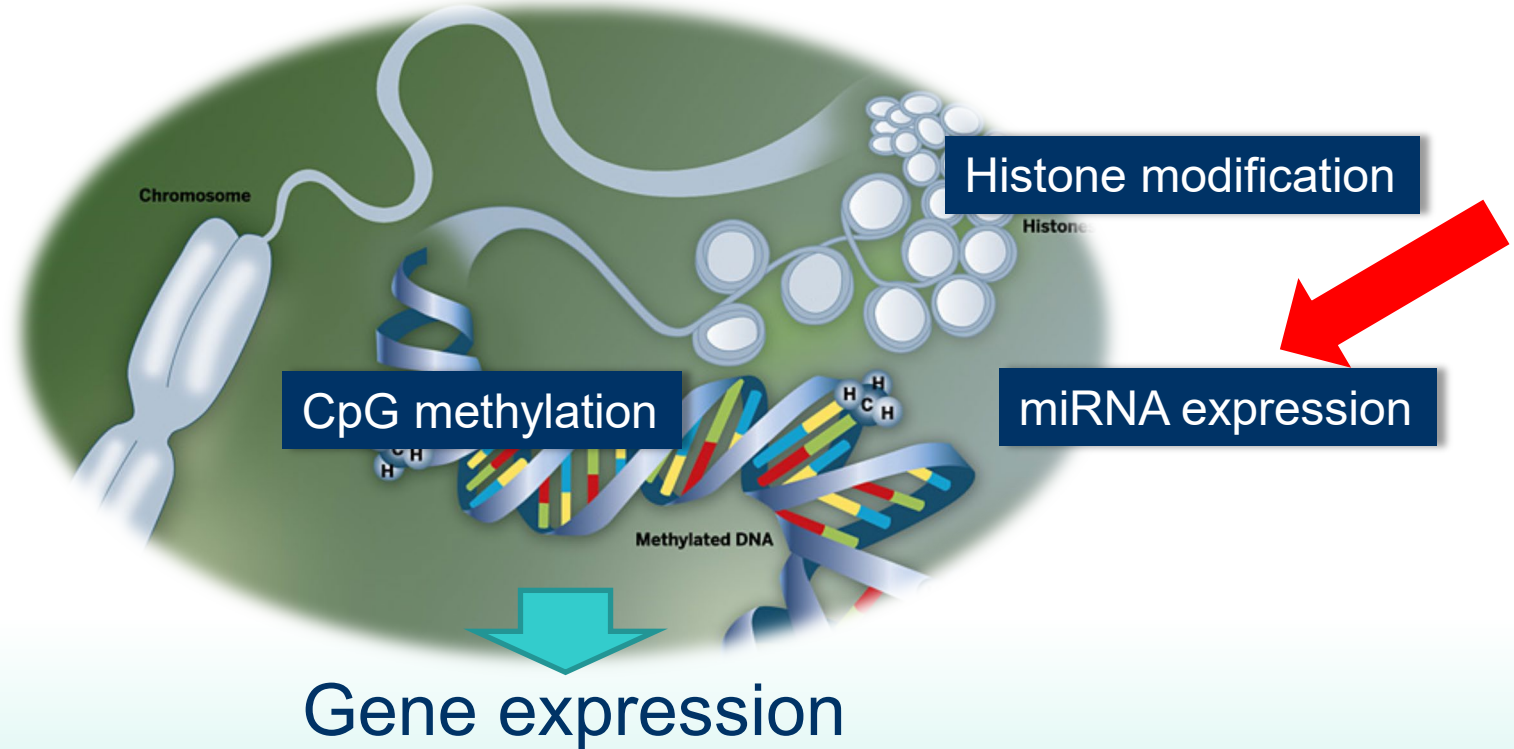
Identify concentrations at which contaminants induce epigenetic effects...

with biological plausibility as causal factors in infant outcomes

150 175 200
)

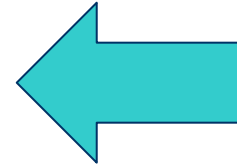


Do Epigenetic Mechanisms Underlie Health Effects Associated with Early Life Exposure?

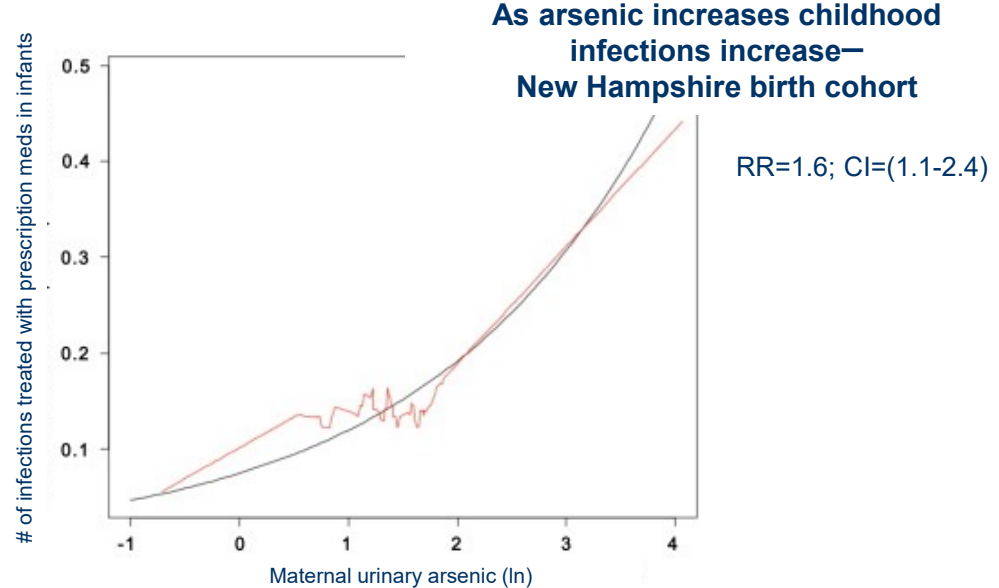


Early Life Health Effects of Inorganic Arsenic

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Epigenetic Effects of Prenatal iAs Exposure: Potential Reprogramming by iAs Early in Life

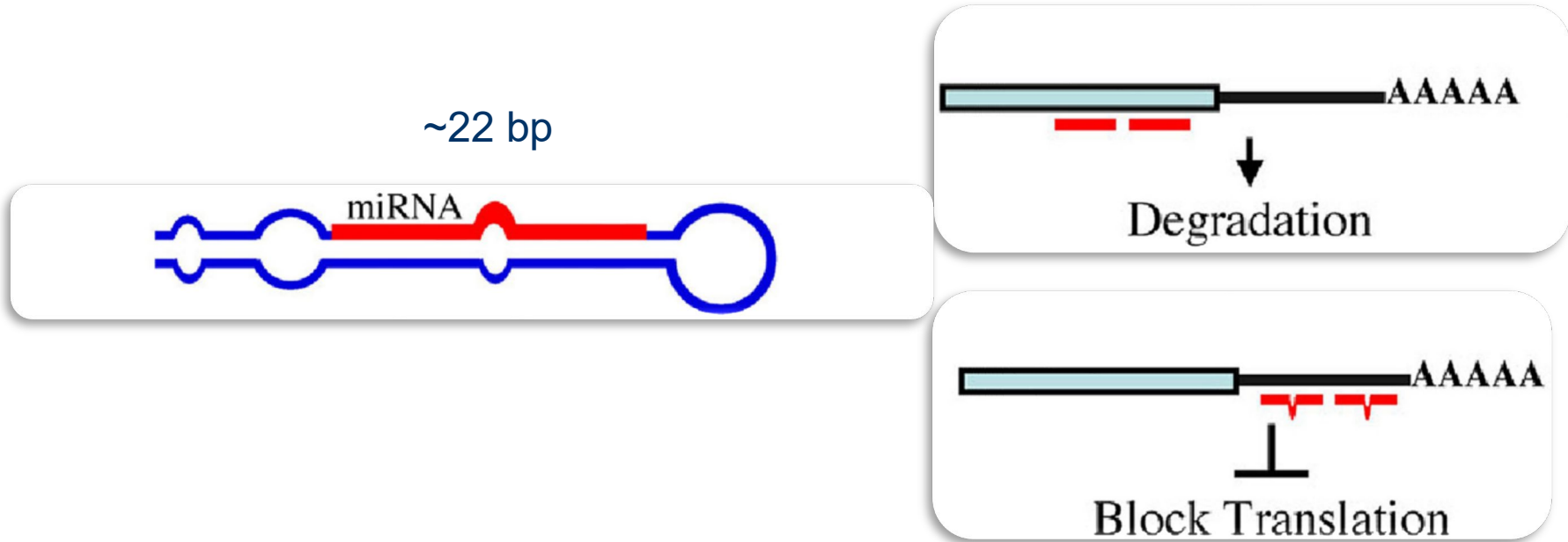


Environmental Research 126 (2013) 24–30

The overall average drinking water As concentration was 5.2 $\mu\text{g/L}$ (range 0.01–67.5 $\mu\text{g/L}$).

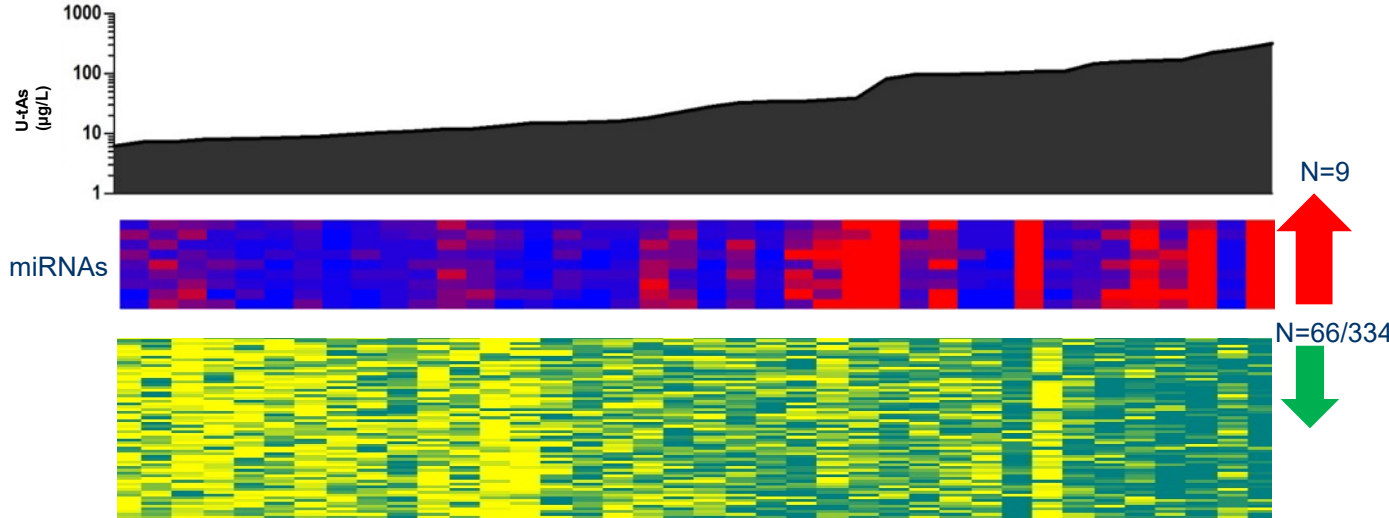


Epigenetic Effects of Prenatal iAs Exposure: Potential Reprogramming by iAs Early in Life



Epigenetic Effects of Prenatal iAs Exposure: Potential Reprogramming by iAs Early in Life

Infants 1-40



Innate and adaptive immune response genes are enriched:

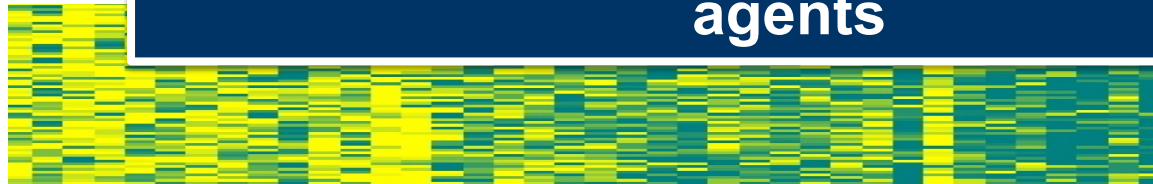
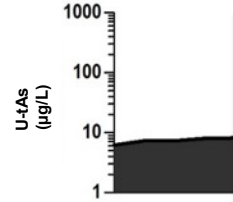
Toll like receptors:
TLR5, TLR9
Interferon gamma pathway

Environmental and Molecular Mutagenesis 55:196–208 (2014)



Epigenetic Effects of Prenatal iAs Exposure: Potential Reprogramming by iAs Early in Life

Infants 1-40



miRNAs REGULATE the expression of innate/adaptive immune response genes

Potential role in response to infectious agents

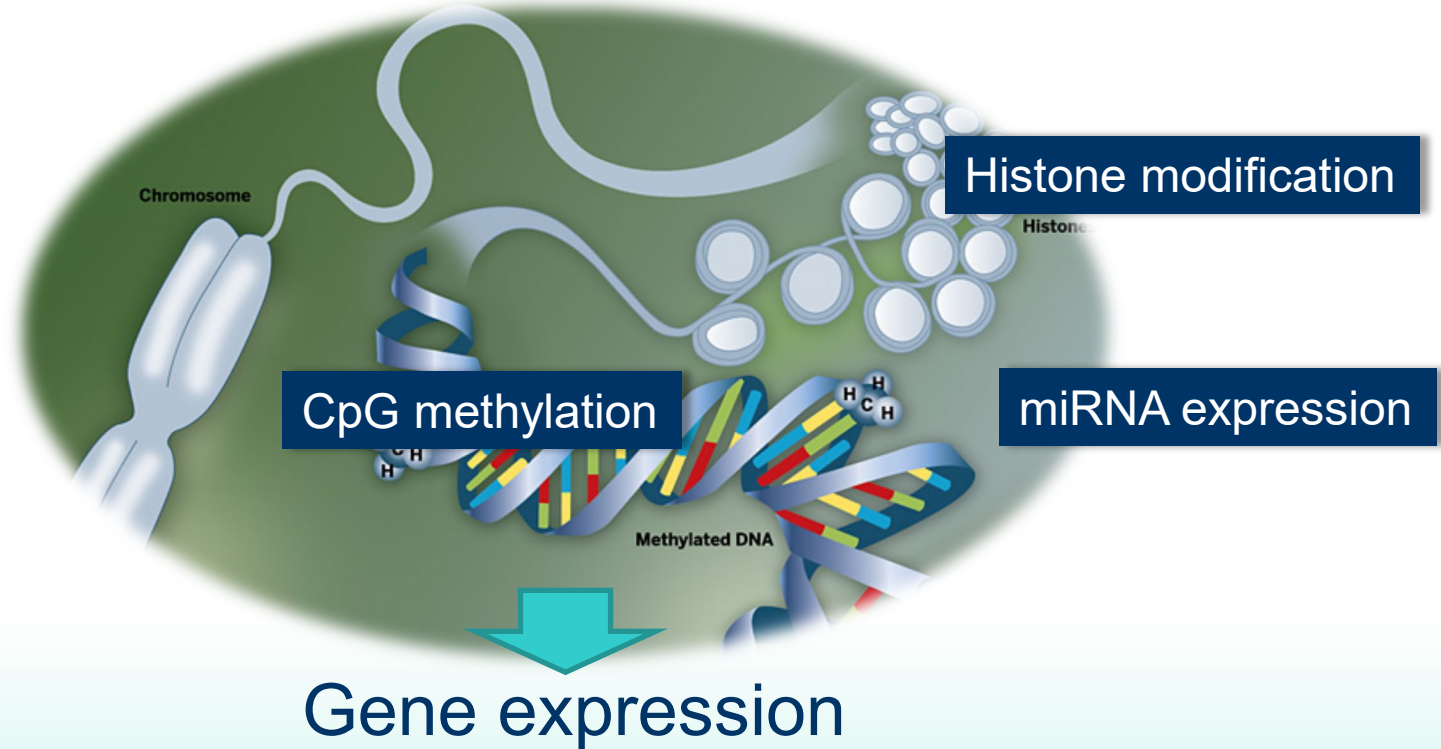
and adaptive immune response genes are enriched:

receptors: TLR5, TLR9
cytokine receptor gamma pathway

Environmental and Molecular Mutagenesis 55:196–208 (2014)



Do Epigenetic Mechanisms Underlie Health Effects Associated with Early Life Exposure?



Summary

- Epigenetic mechanisms may tie early life arsenic exposure to early and later in life health outcomes
 - miRNAs
 - CpG methylation
- The placenta is a key target organ for developmental exposure to arsenic
- Preconception arsenic exposure is tied to offspring health in mice



References

- Laine, Jessica E. et al. 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. <https://doi.org/10.1289/ehp.1307476>
- Rager, Julia E, et al. 2014. Prenatal Arsenic Exposure and the Epigenome: Altered microRNAs Associated with Innate and Adaptive Immune Signaling in Newborn Cord Blood. *Environmental and Molecular Mutagenesis* Vol 55(3)(April):196-208. <https://doi.org/10.1002/em.21842>
- Rojas, Daniel, et al. 2015. Prenatal Arsenic Exposure and the Epigenome: Identifying Sites of 5-methylcytosine Alterations that Predict Functional Changes in Gene Expression in Newborn Cord Blood and Subsequent Birth Outcomes, *Toxicological Sciences*, Volume 143, Issue 1(January): 97–106. <https://doi.org/10.1093/toxsci/kfu210>



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UNC Institute for Environmental Health
Solutions & UNC Superfund Research
Program



Environmental Epigenetics
Laboratory

