Health Effects of Metal Exposures

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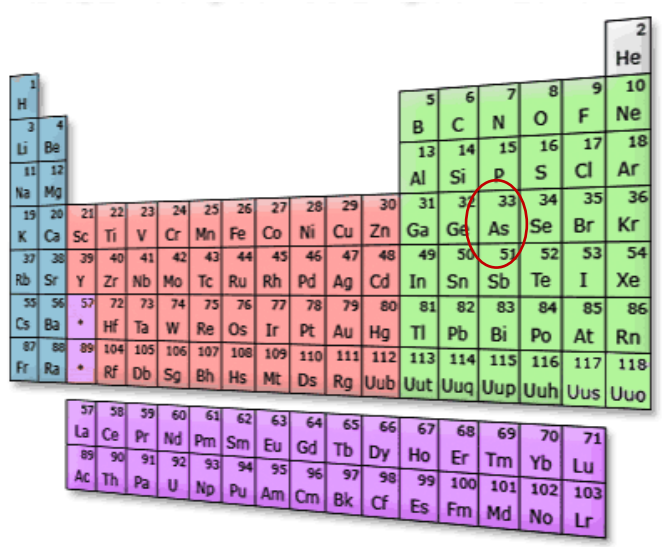
Environmental Health Research in Bangladesh

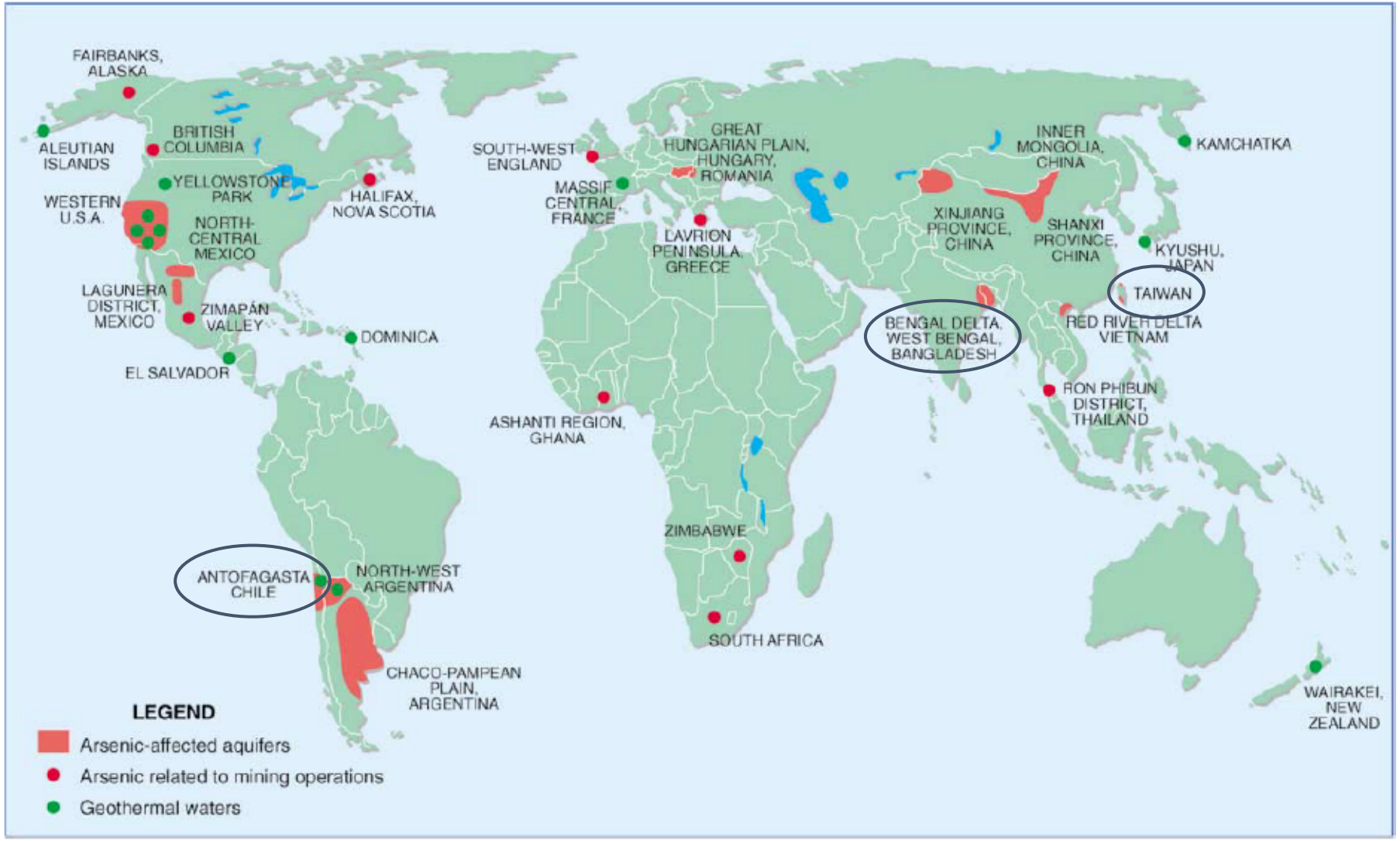
- Health Effects of Arsenic Longitudinal Study (HEALS; PI Ahsan)
- Bangladesh Vitamin E and Selenium Trial (BEST; PI Ahsan)
- Bangladesh Environmental Research in Children's Health (BiRCH; PI Argos)

Inorganic Arsenic

- Naturally occurring element (metalloid) widely distributed in environment
- 20th most abundant element in the Earth's crust
- Human exposure to arsenic through water, food, soil, dust and air
- Moderate-to-high doses of arsenic exposure via water associated with adverse health effects
- Epidemiologic evidence sparse
  - In low-dose range
  - Characterizing exposure from sources other than water



Global Distribution of Arsenic in Drinking Water



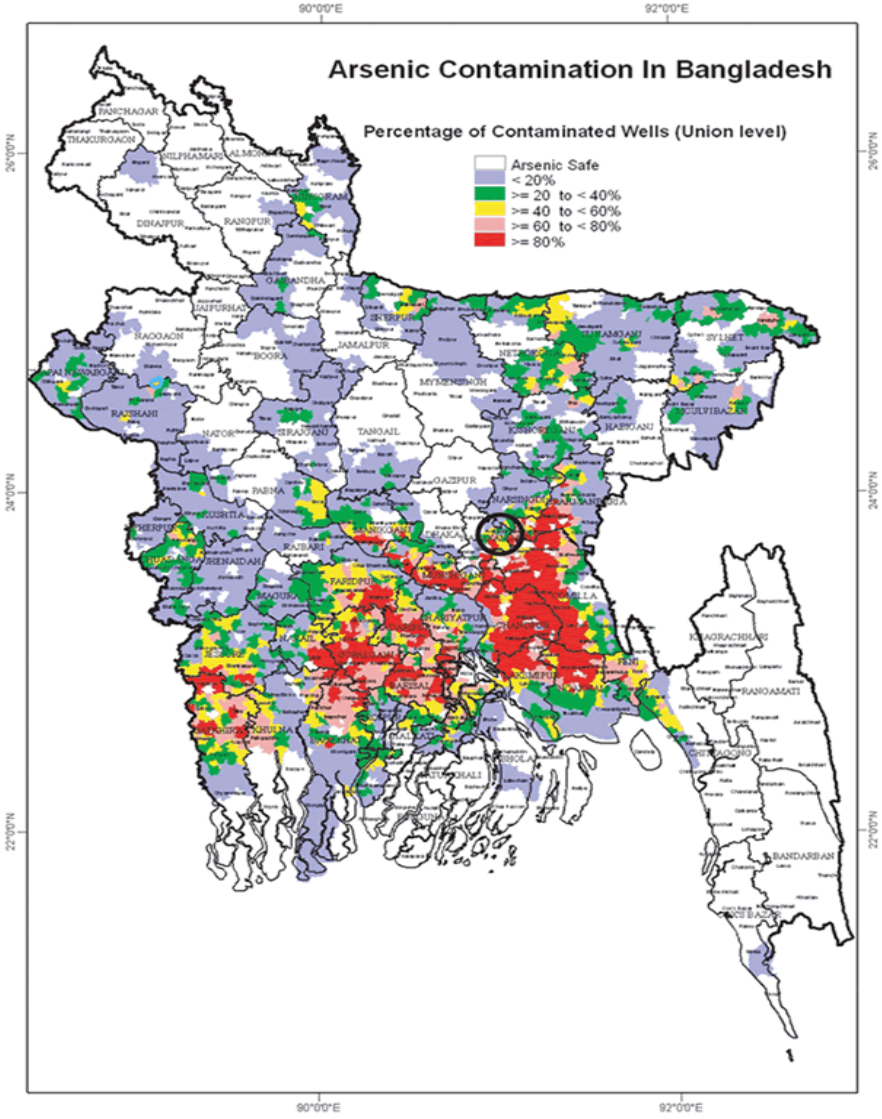
Smedley & Kinniburgh (2002) *Applied Geochemistry* 17:517-68.

Arsenic Exposure in Bangladesh

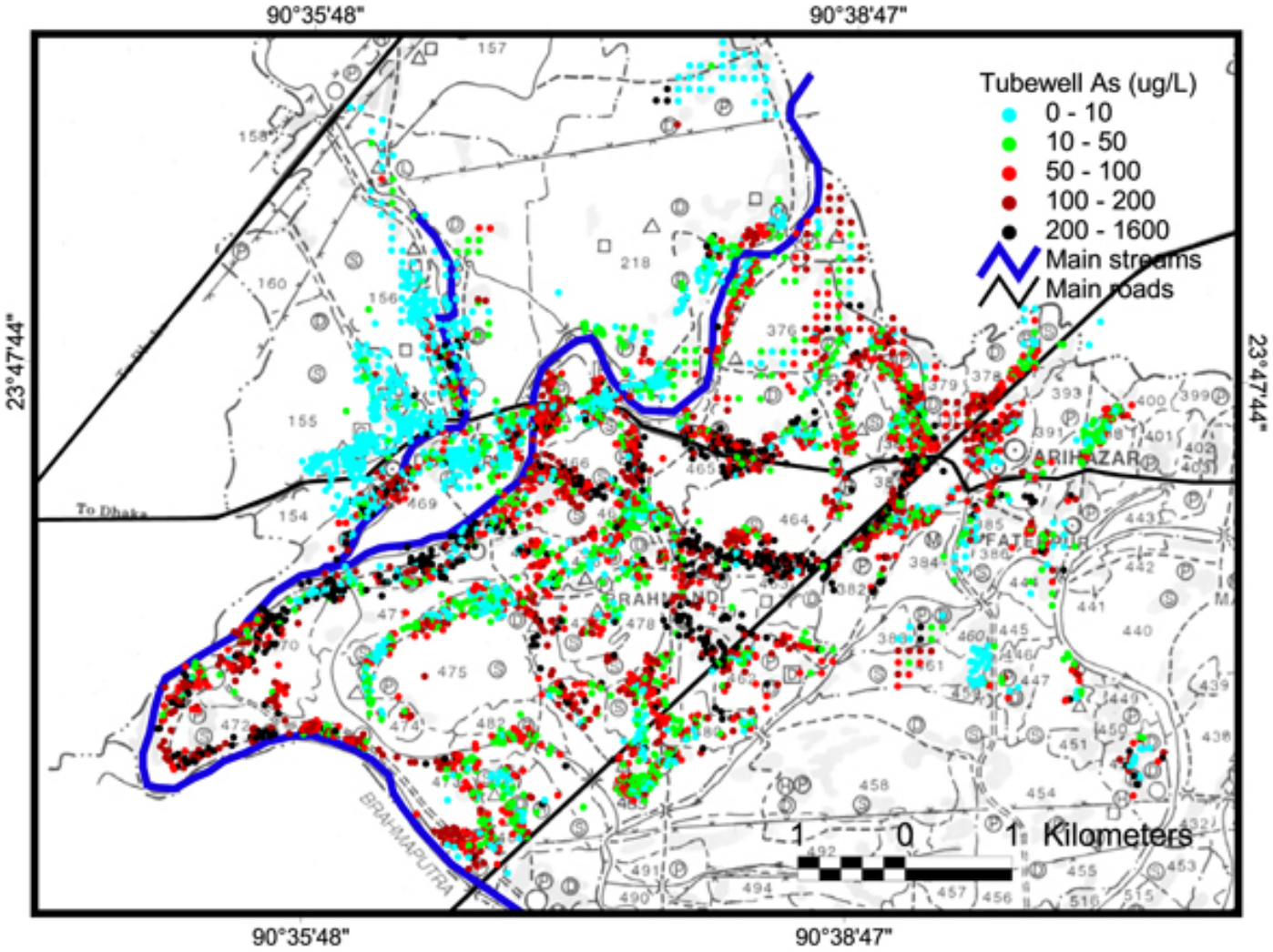
- Magnitude of affected population most severe in Bangladesh
- Hand-pumped tubewells installed to provide pathogen-free groundwater beginning in 1970s
- Groundwater in Bangladesh naturally contaminated with high levels of arsenic
- Arsenic in groundwater discovered only after decades of exposure and an epidemic of skin lesions, a hallmark characteristic of arsenic toxicity

Magnitude of Public Health Issue in Bangladesh

- British Geological Survey study of wells in Bangladesh
  - 27% of well samples >50 µg/L → 28-35 million individuals
  - 46% of well samples >10 µg/L → 46-57 million individuals (est. 2000)



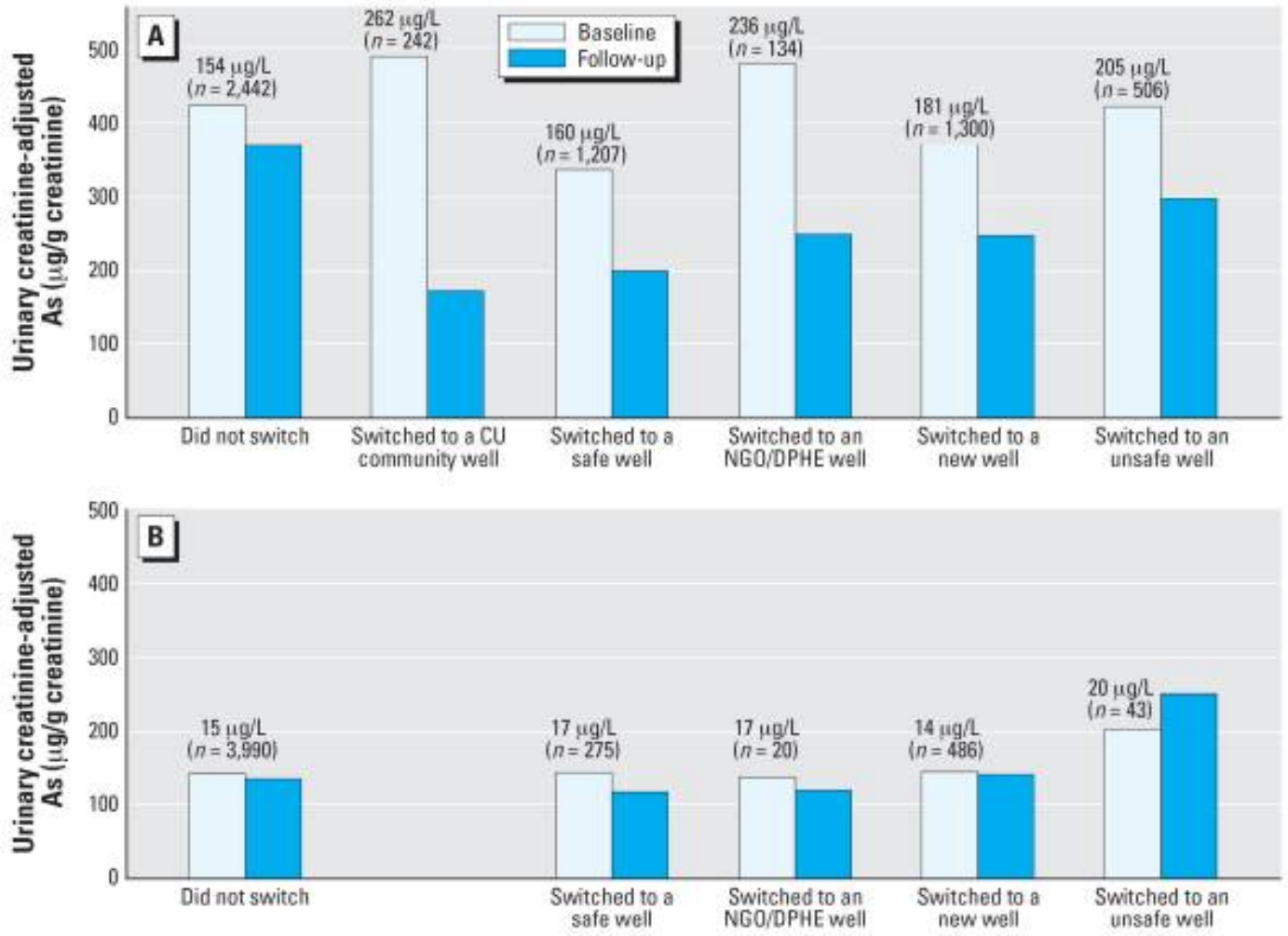
Geographic Well Arsenic Distribution in Araihazar



Exposure Mitigation Campaigns

- All wells in study area tested and labeled with their arsenic concentration
- Community educational programs to promote well switching
- Installation of deep community wells
- Distribution of SONO filters

Well Switching



Chen Y et al (2007) *Environ Health Perspect* 115(6):917-923.

Health Effects of Chronic Arsenic Exposure

- Skin lesions
- Non-melanoma skin cancers
- Internal cancers:
  - Bladder
  - Kidney
  - Lung
  - Liver
  - Prostate
- Developmental effects
- Neurological effects
- Hypertension
- Cardiovascular disease
- Pulmonary disease
- Peripheral vascular disease
- Mortality
- Reproductive effects
- Diabetes

Arsenic Exposure and Mortality

	All-cause mortality*		Chronic-disease mortality*	
	Deaths	HR (95% CI)	Deaths	HR (95% CI)
Arsenic (µg/L) in well water				
0·1–10·0	74	1·00	58	1·00
10·1–50·0	90	1·34 (0·99–1·82)	69	1·33 (0·94–1·87)
50·1–150·0	98	1·09 (0·81–1·47)	83	1·22 (0·87–1·70)
150·1–864·0	131	1·68 (1·26–2·23)	101	1·68 (1·21–2·33)
Arsenic dose (µg per day)				
0·041–35·0	87	1·00	66	1·00
35·1–163·0	97	1·10 (0·83–1·47)	80	1·21 (0·88–1·67)
163·1–401·0	91	1·09 (0·81–1·46)	76	1·22 (0·88–1·71)
401·1–4898·0	118	1·54 (1·17–2·04)	89	1·58 (1·15–2·18)
Total arsenic in urine (µg/g)				
7·0–105·0	83	1·00	64	1·00
105·1–199·0	96	1·07 (0·80–1·43)	80	1·17 (0·84–1·62)
199·1–352·0	100	1·22 (0·91–1·63)	83	1·37 (0·98–1·90)
352·1–5000·0	105	1·45 (1·09–1·94)	77	1·47 (1·05–2·06)

Data are number or HR (95% CI). *Multivariate estimates were adjusted for age, sex, body-mass index, systolic blood pressure, education, and smoking status.

Table 2: **Hazard ratio (HR) for mortality in participants in relation to baseline arsenic exposure**

- **All-cause mortality**
  - 21% could be attributed to arsenic exposure from drinking water at concentrations >10 µg/L
- **Chronic disease mortality**
  - 24% could be attributed to arsenic exposure from drinking water at concentrations >10 µg/L

Argos et al. (2010) *Lancet* 376(9737):252-8

Arsenic Exposure and Pregnancy Outcomes

Crude and adjusted odds ratios (95% CIs) for the associations between prenatal arsenic exposure and adverse pregnancy outcomes.

Model	Urinary total arsenic (μg/g creatinine)		
	17 – 555	556 – 3712	P-value
Any adverse pregnancy outcome			
Unadjusted	1 (ref)	1.53 (1.00, 2.35)	0.05
Adjusted for maternal age	1 (ref)	1.58 (1.02, 2.42)	0.04
Fully adjusted[a]	1 (ref)	1.59 (1.02, 2.46)	0.04
Stillbirth/spontaneous abortion			
Unadjusted	1 (ref)	1.50 (0.92, 2.45)	0.10
Adjusted for maternal age	1 (ref)	1.51 (0.93, 2.47)	0.10
Fully adjusted[a]	1 (ref)	1.57 (0.96, 2.56)	0.07
Stillbirth			
Unadjusted	1 (ref)	2.41 (1.00, 5.85)	0.05
Adjusted for maternal age	1 (ref)	2.41 (0.99, 5.85)	0.05
Fully adjusted[b]	1 (ref)	2.50 (1.04, 6.01)	0.05
Spontaneous abortion			
Unadjusted	1 (ref)	1.26 (0.72, 2.20)	0.41
Adjusted for maternal age	1 (ref)	1.27 (0.72, 2.24)	0.42
Fully adjusted[a]	1 (ref)	1.33 (0.76, 2.32)	0.32
Therapeutic/elective abortion			
Unadjusted	1 (ref)	1.67 (0.75, 3.73)	0.21
Adjusted for maternal age	1 (ref)	1.69 (0.75, 3.81)	0.21
Fully adjusted[a]	1 (ref)	1.58 (0.70, 3.56)	0.27

[a] Adjusted for maternal age (years), maternal education (years), BEST treatment assignment, and skin lesion severity.

[b] Adjusted for maternal age (years), maternal education (years), BEST treatment assignment, skin lesion severity, parity, and previous stillbirth.

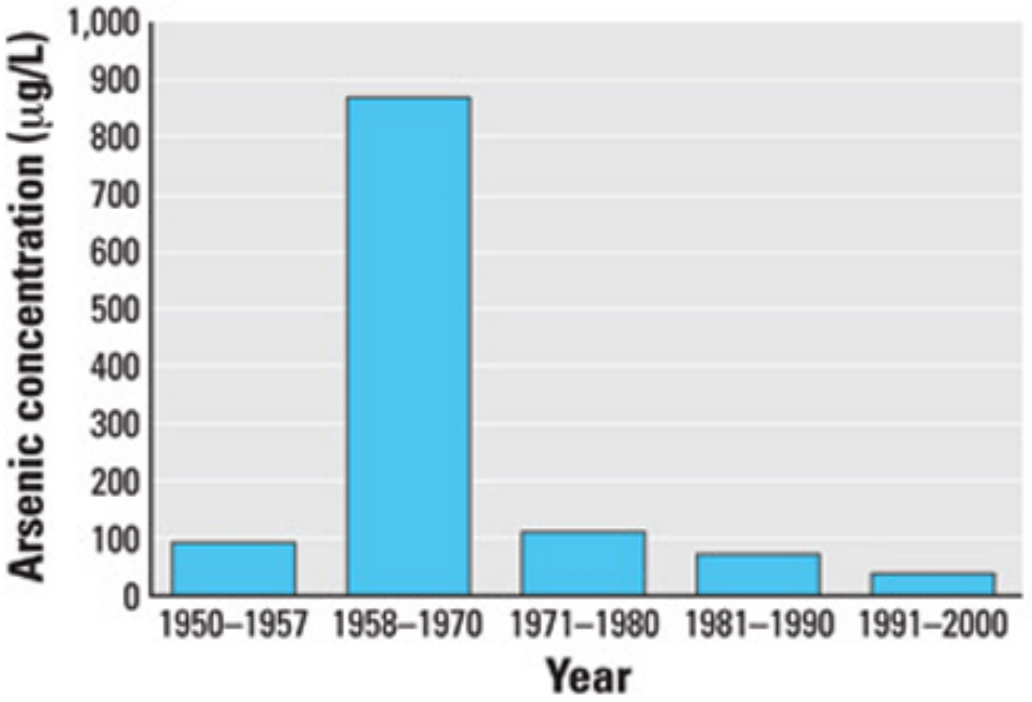
Shih et al. *Environmental Research* 2017. 158 :456–461

Developmental Origins of Health and Disease (DOHaD)

- Arsenic implicated in development of variety of diseases in adults
- Critical developmental period, beginning in utero and continuing into early postnatal life, uniquely sensitive to environmental insults
- Early-life exposure might permanently change the body's structure, metabolism, and physiology, and hence promote health or diseases in later stages of life

Early-life Arsenic Exposure and Cancer

Much of what we have learned about the carcinogenic effects of in utero and early arsenic exposure in humans has come from ecologic studies in Antofagasta, Chile



Kidney Cancer Mortality

TABLE 3. Standardized Mortality Ratios[a] for Kidney Cancer Mortality Among Young Adults in Antofagasta and Mejillones[b] Aged 30–39 Years Who Were Born During and Just Before the High-Exposure Period, and for Ages 40 and Above Who Were Born Before 1950 and Would Not Have Had Early Life Exposure

Age (Years)	Birth Cohort	No. Observed Deaths	No. Expected Deaths	SMR (95% CI)
Men				
30–39	1950–1970	4	0.71	5.63 (1.52–14.4)
40+	Before 1950	103	38	2.68 (2.19–3.26)
Women				
30–39	1950–1970	4	0.42	9.52 (2.56–24.4)
40+	Before 1950	84	21	3.91 (3.12–4.84)
Total				
30–39	1950–1970	8	1.13	7.08 (3.05–14.0)
40+	Before 1950	187	60	3.12 (2.69–3.61)

[a]Standardized mortality ratios with expected mortality estimated from the rest of Chile excluding Region II.

[b]Antofagasta and Mejillones used the same water source, containing about 870 μg/L of arsenic, during the period 1958–1970.

- Association of in utero and early childhood arsenic exposure with kidney cancer mortality
- 1950-1970 birth cohort exposed in utero or early childhood
- Before 1950 birth cohort exposed later in childhood or adolescence

Yuan et al. (2010) *Epidemiology* 21(1):103-8.

Paradigm Shift for Arsenic Epidemiology

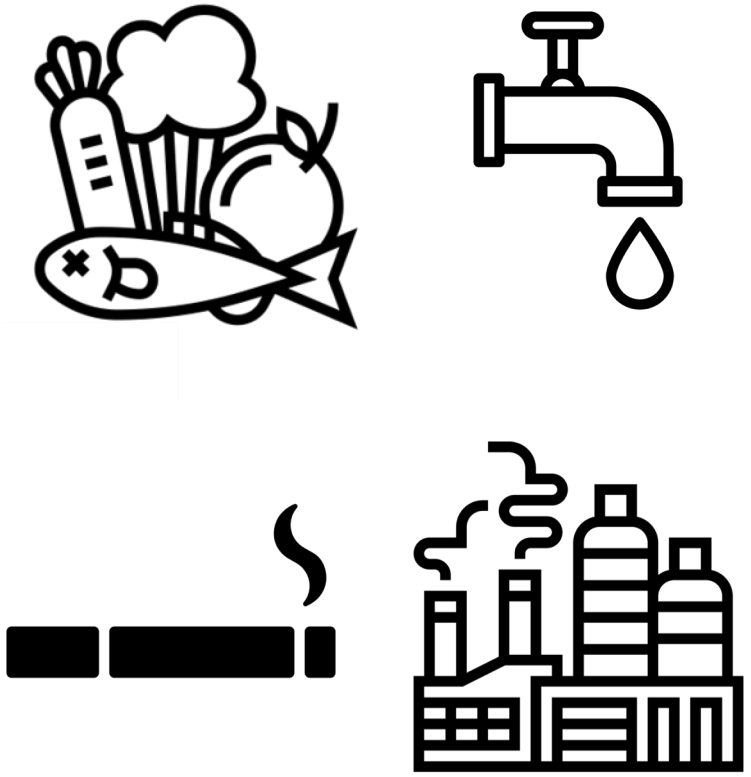
- Change in focus of hypotheses
- Chronic disease risk may be programmed during sensitive periods in utero and in early-life
- Prevention efforts for adult-onset diseases may be better targeted to sensitive periods in pregnancy, early childhood, or adolescence

BiRCH Cohort

- Bangladesh Environmental Research in Children's Health cohort (BiRCH; NIH R01 ES024423)
- 500 mother-child pairs
  - Children aged 5-7 years at enrollment in 2014 -2016
  - Data sources include questionnaire data, clinical assessment, biological specimens

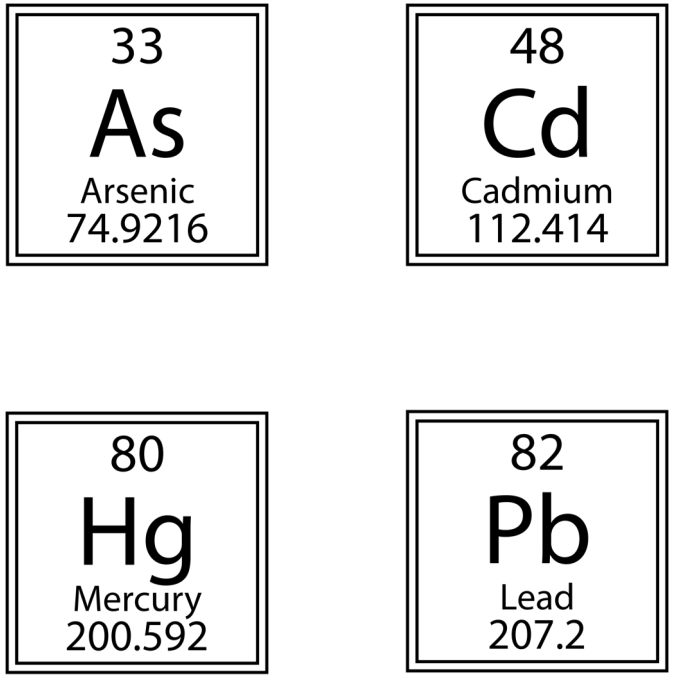
Metals and Biological Aging

- Global public health concern for toxic metal exposures that are ubiquitous in the environment
    - Arsenic, cadmium, lead, and mercury exposures in the general population
- Emerging evidence that environmental factors influence biological aging, such as epigenetic age and telomere shortening



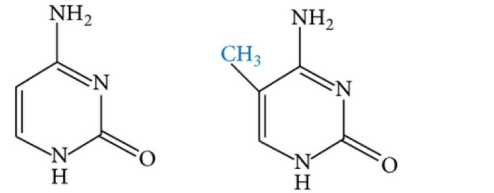
Objective

To evaluate the cross-sectional associations of toenail metal concentrations with markers of biological aging in Bangladeshi children aged 5-7 years

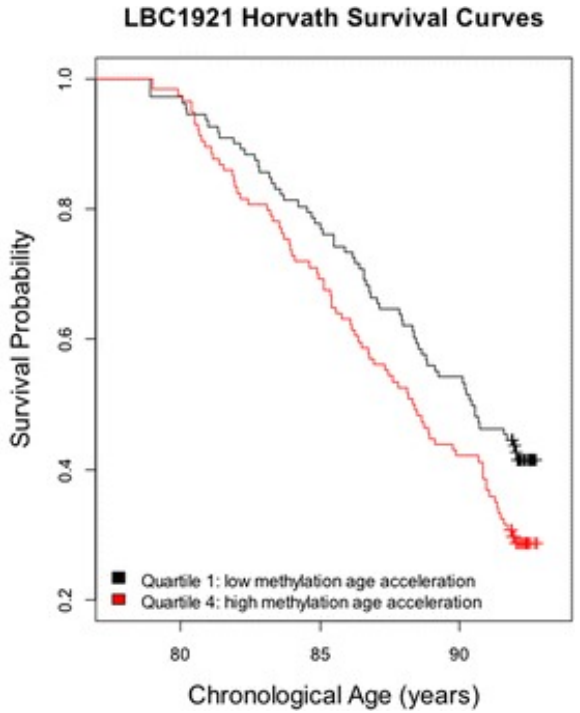


Epigenetic Clock

- At the molecular level, aging described as the accumulation of cellular damage, leading to structural and functional abnormalities and decrease the regenerative capacity of cells
- Biological aging reported to be a risk factor for the development of age-related diseases and increased mortality
- DNA methylation age
  - Sets of DNA methylation markers (CpG sites) representing biological aging
  - Highly correlated with chronological age
  - Accelerated by environmental exposures
  - Higher DNA methylation age relative to chronological age associated with CVD, cancer, and mortality

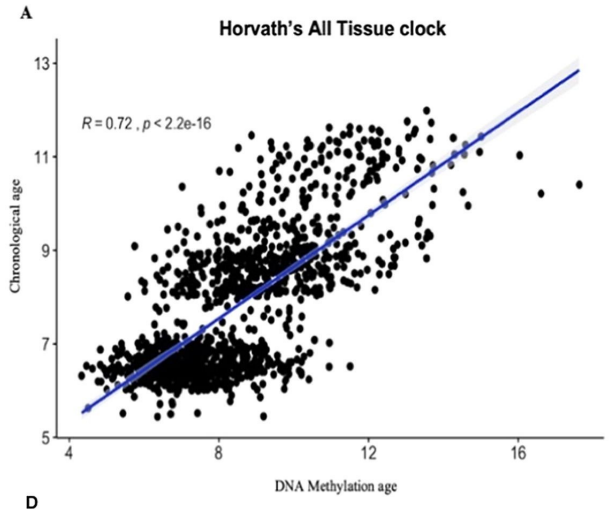


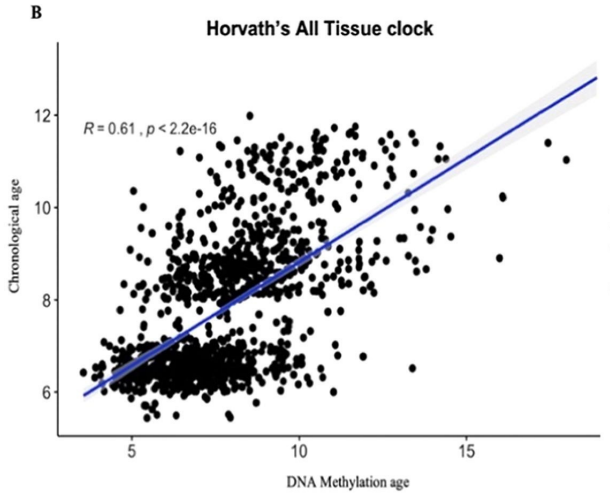
Cytosine 5-Methylcytosine

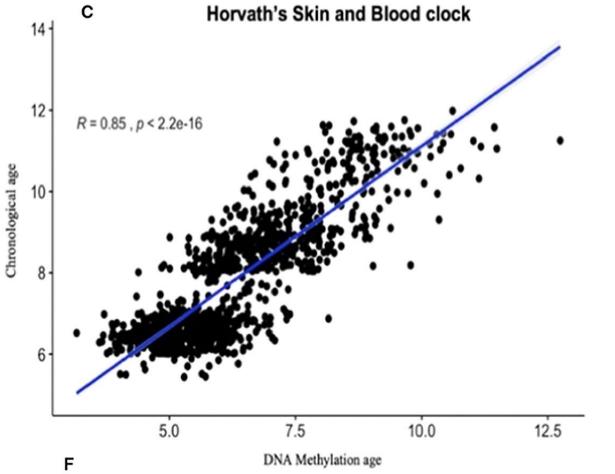


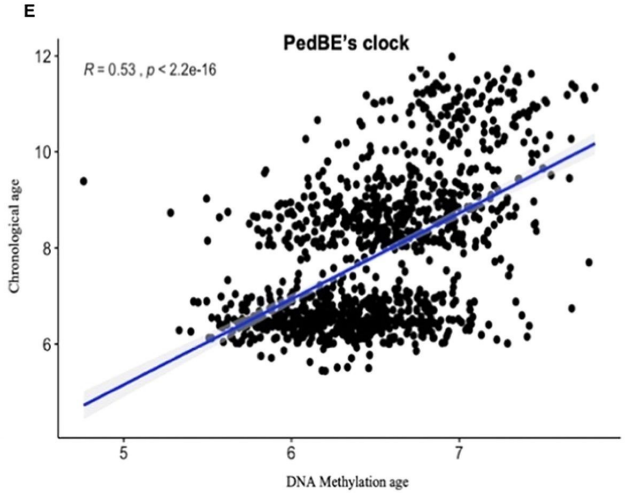
Marioni R et al. Genome Biol. 2015; 16(1):25.

Epigenetic Age Measures in Children









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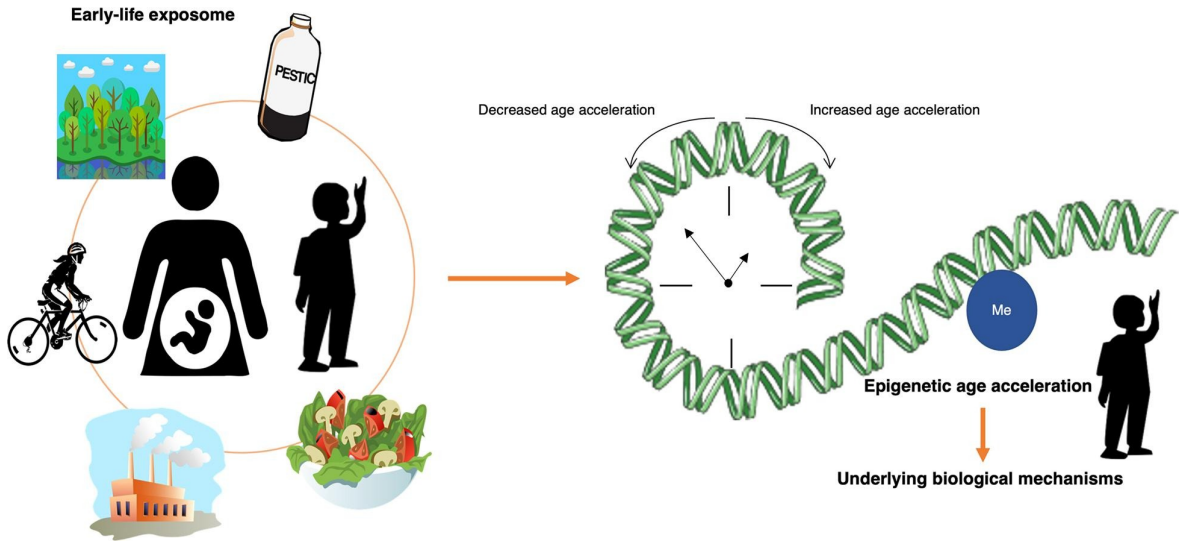
Clocks	Mean	Standard deviation
Horvath's All Tissue clock	8.532	2.014
Horvath's All Tissue clock (online calculator)	7.814	2.081
Horvath's Skin and Blood clock	6.311	1.474
Wu's clock	4.075	0.749
PedBE's clock	6.503	0.455
Chronological age	7.835	1.543

de Prado-Bert et al. Environ Int. 2021; 155:106683.

Epigenetic Clock and Children's Health

- Aging continuous process already starting in early-life
- Age-associated DNA methylation changes occur more rapidly in children than adults (Alisch et al. 2012)



de Prado-Bert et al. Environ Int. 2021; 155:106683.

Limited Epidemiologic Research in Children

Table 2
ExWAS* of prenatal and childhood exposures vs. age acceleration adjusted for blood cell type proportions (main model).

				ExWAS*	
	Exposure	**Exposure family**	**Units**	**Estimate (95% CI) [a]**	**P-value**
Prenatal	Maternal tobacco smoking	Tobacco smoke	No vs. Yes	0.14 (0.02, 0.26)	0.025
Childhood	Indoor PM_{abs}	Indoor air	ug/m^3	0.07 (0.02, 0.12)	0.003
	Parental smoking	Tobacco smoke	Neither vs. Both	0.15 (0.01, 0.29)	0.036
	Dimethyl dithiophosphate (DMDTP)	OP Pesticides	Undetected vs. Detected (adjusted for creatinine)	−0.13 (−0.24, −0.02)	0.017
	Polychlorinated biphenyl-138 (PCB-138)	OCs	ng/g (adjusted for lipids)	−0.07 (−0.14, 0.01)	0.037

Note: ExWAS = exposome-wide association study; PM_{abs} = Particulate Matter Absorbance, DMDTP = Dimethyl dithiophosphate; OP Pesticides = Organophosphate Pesticides; PCB-138 = Polychlorinated biphenyl-138; OCs = Organochlorine compounds; IQR = Interquartile range. *Results are presented only for the exposures with nominal significance (p value < 0.05) in the ExWAS adjusted for: child's sex, cohort, self-reported maternal education, self-reported ancestry and maternal age in years. The analyses were conducted in 1,173 children from the HELIX subcohort. [a]Coefficient estimates are given in age acceleration effect change for each IQR increase in continuous exposure variables, or relative to the reference category in binary and categorical variables.

de Prado-Bert et al. Environ Int. 2021; 155:106683.

Study Measures

- Child's toenail clippings assessed for metal concentrations
    - Inductively coupled plasma mass spectrometry (ICP-MS) at Dartmouth College Trace Element Analysis Core
- Blood leukocyte DNA assessed for DNA methylation
    - Illumina EPIC array at the University of Chicago Institute for Population and Precision Health Laboratory
    - DNA methylation data preprocessing and quality control completed using ENmix R package
- Three epigenetic clock measures estimated: Horvath, Hannum, and PhenoAge

Participant Characteristics

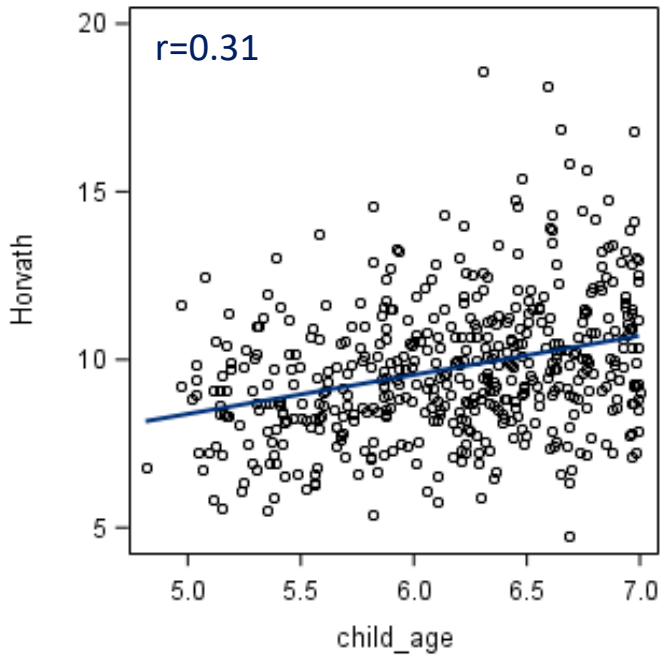
Complete case analysis conducted using 491 participants with available data on toenail metal concentrations, epigenetic age, and covariates

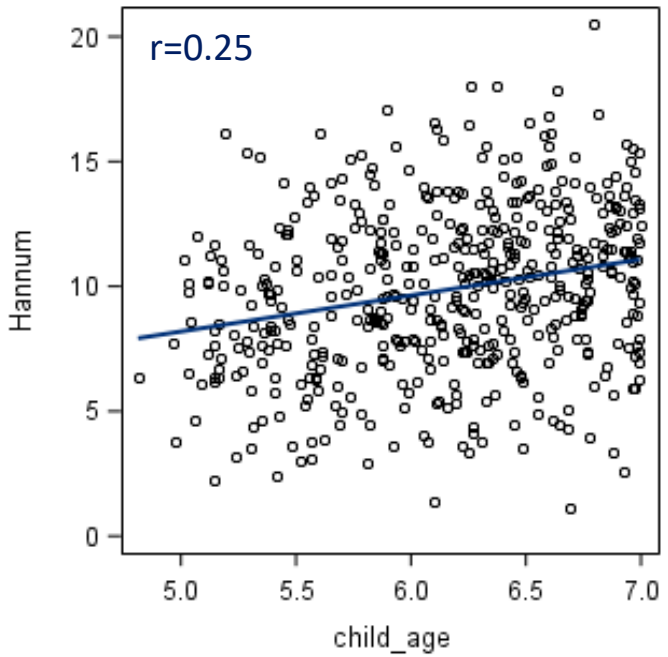
Toenail metal concentrations, µg/g	Median (IQR)
Arsenic	1.69 (2.04)
Cadmium	0.11 (0.12)
Lead	1.92 (1.68)
Mercury	0.19 (0.17)

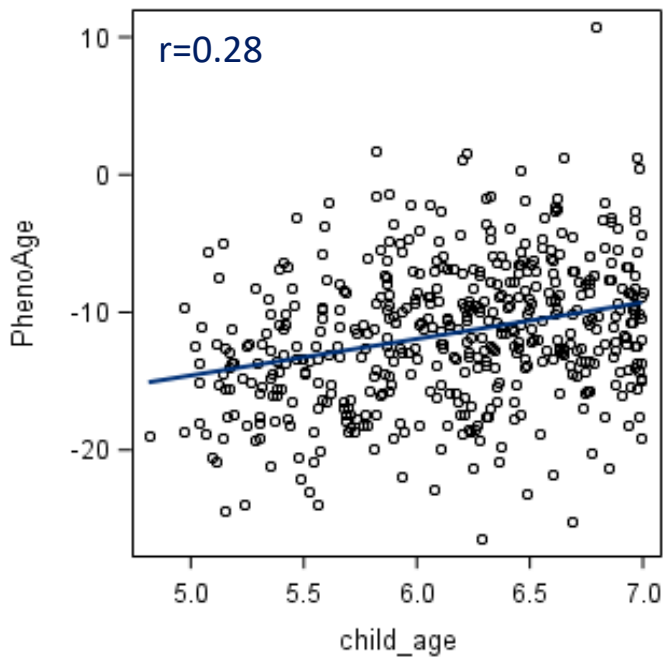
Characteristics of participants	
Sex	
Male	248 (50.5%)
Female	243 (49.5%)
Mean chronological age, years	6.16 (0.55)

Spearman correlation coefficients			
Cd	0.22		
Pb	0.19	0.43	
Hg	0.27	0.21	0.19
	As	Cd	Pb

DNA Methylation Age

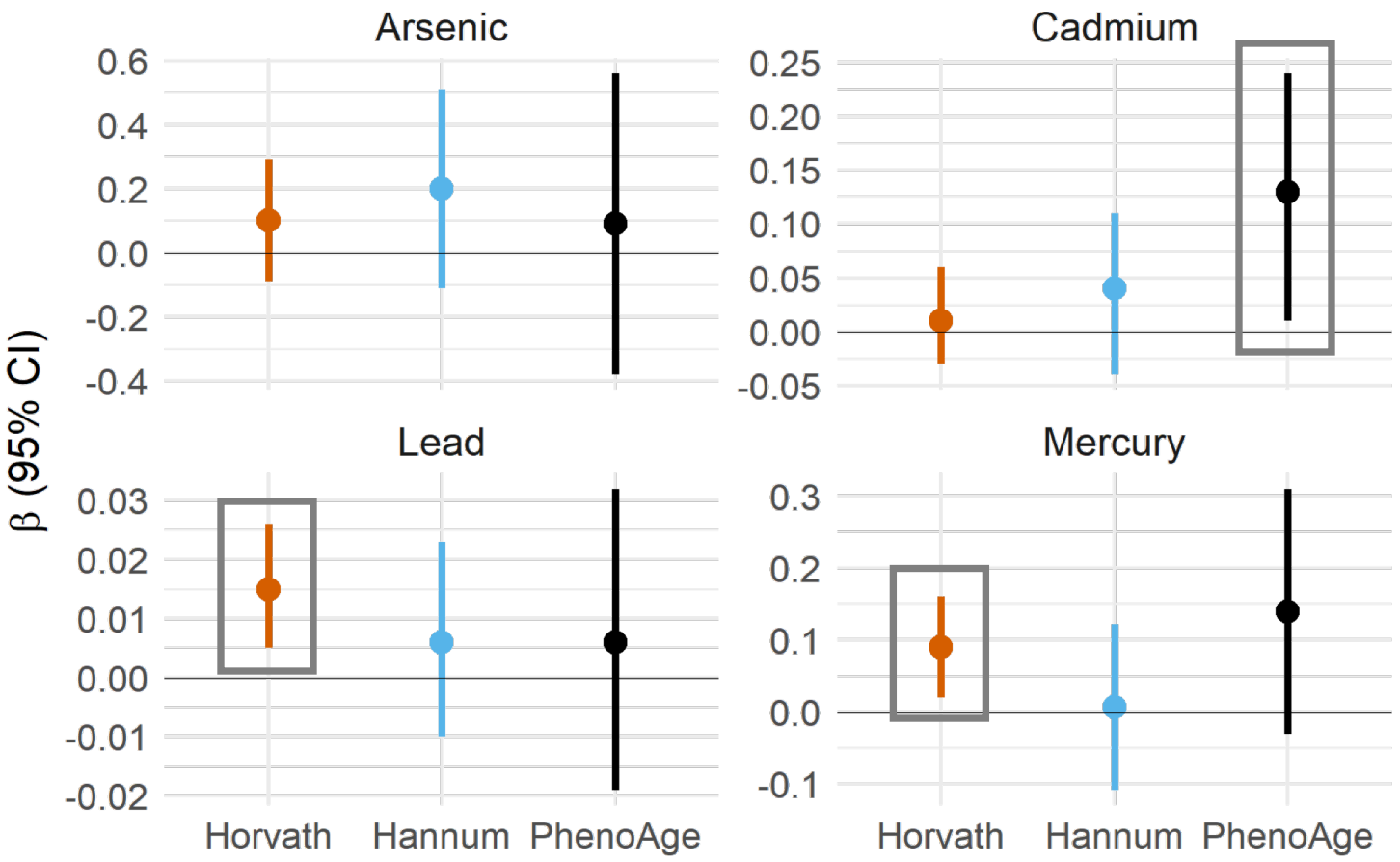






Spearman correlation coefficients		
Hannum	0.47	
PhenoAge	0.47	0.55
	Horvath	Hannum

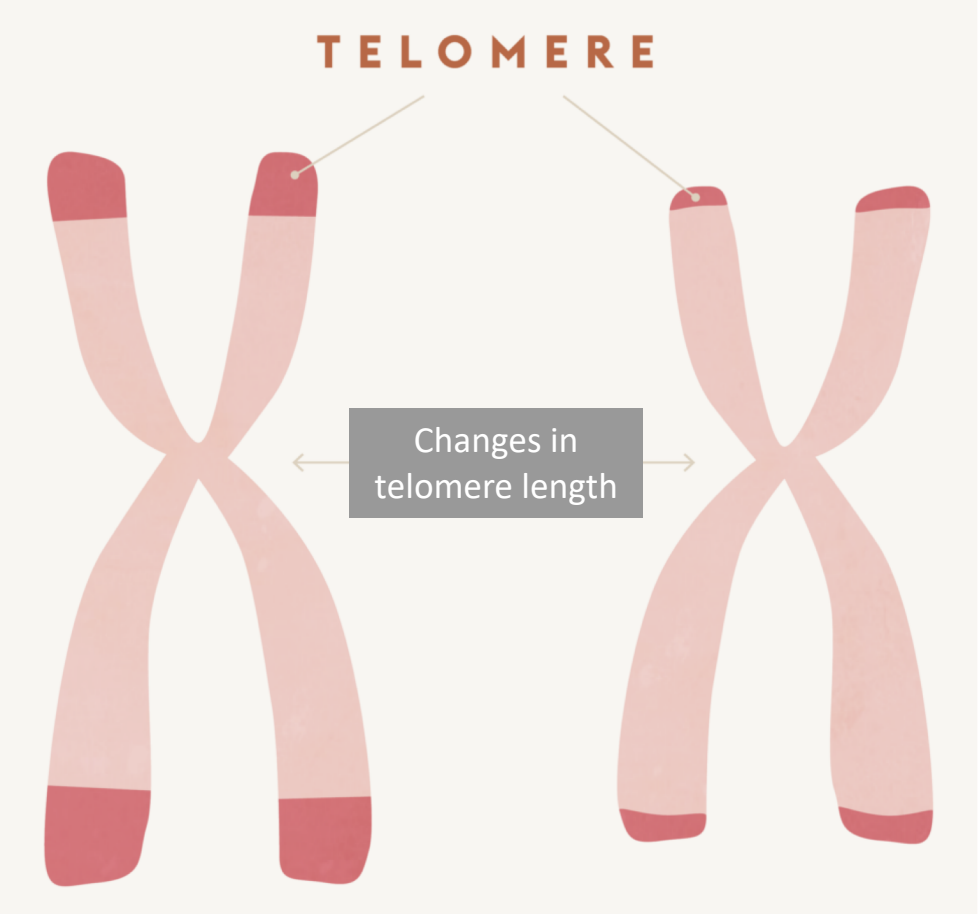
Toenail Metals and Epigenetic Age



Effect estimate for IQR increase in toenail metal concentration
Models adjusted for age, sex, body mass index, maternal education, and environmental tobacco smoke

Telomere Length

- Preserve genetic material during cell division and protect it from damage
- Shortening of telomeres occurs over time with each cell division
- Shortening eventually leads to cellular senescence/apoptosis
- Telomere length ~ common chronic and age-related diseases, (e.g. CVD, obesity, metabolic health, cancer)



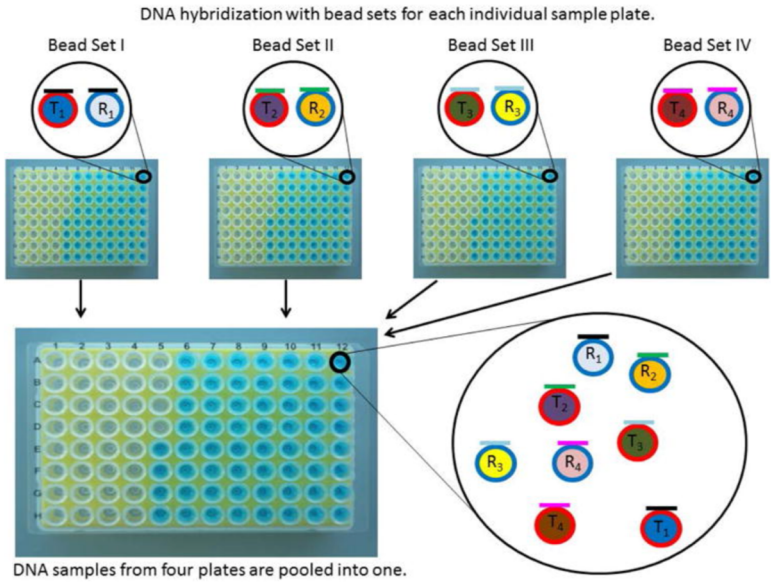
Repetitive sequences of DNA & binding proteins form a protective cap on chromosome ends

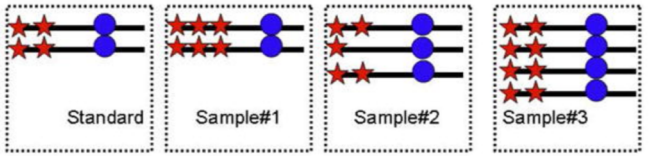
Telomeres and Children's Health

- Early childhood is a critical period for telomere biology
    - Rapid decline in telomere length due to a large turnover of highly proliferative cells
- Telomere length may represent a biomarker of susceptibility to disease in younger populations
- Environmental insults in early life may have a more considerable impact on telomere attrition than in adulthood

Assessment of Telomere Length

- Relative telomere length (RTL) was determined by a novel high-throughput Luminex assay (Jasmine et al 2018; Kibriya et al 2014)
- DNA is hybridized to sequence-specific probes for the telomere repeat and reference gene sequences
- Telomere and reference gene signals are amplified and detected using Luminex
- RTL is calculated as telomere signal (red stars) divided by reference gene signal (blue circles) and normalized to a standard signal





	Standard	Sample#1	Sample#2	Sample#3
MFI of TEL	4	6	5	8
MFI of ALK	2	2	3	4
Quantification TEL	1	6/4=1.5	5/4=1.25	8/4=2
Quantification ALK	1	2/2=1	3/2=1.5	4/2=2
TQI= TEL/ALK	1	1.5/1=1.5	1.25/1.5=0.83	2/2=1

Arsenic and Mercury Associated with Shorter Telomere Length

Associations of log2-transformed child toenail metals and relative telomere length among participants

	N	Adj. covariates[a] b (95% CI)	p-value	Adj. covariates, co-exposures[b] b (95% CI)	p-value
Log_2-As, µg/g	455	-0.022 (-0.033, -0.011)	0.0001	-0.019 (-0.031, -0.007)	0.003
Log_2- Hg, µg/g	455	-0.020 (-0.032, -0.007)	0.0019	-0.015 (-0.028, -0.002)	0.022

[a] Adjusted for child age, sex and father's age at child's birth
[b] Adjusted for co-exposures (eg. As model adj for Hg)

Associations with Telomere Length by Sex

Sex-stratified associations[a] of log2-transformed child toenail metals and relative telomere length among participants, adjusted for covariates and co-exposure

	Males			Females		
	N	b (95% CI)	p-value	N	b (95% CI)	p-value
Log_2-As, µg/g	223	-0.013 (-0.030, 0.004)	0.12	232	-0.026 (-0.042, -0.009)	0.002
Log_2-Hg, µg/g	223	-0.025 (-0.042, -0.007)	0.007	232	-0.004 (-0.022, 0.013)	0.63

[a] Adjusted for child age, father's age at child's birth, with mutual adjustment for co-exposure to As and Hg

Conclusions

- Higher concentrations of toenail lead and mercury independently associated with epigenetic age acceleration based on Horvath DNA methylation age
- Higher concentrations of toenail arsenic and mercury independently associated with shorter telomere length
- Suggests metal exposures may influence biological aging markers in early life
- Evaluating aging during this period might provide new evidence to slow down this process from the beginning and, prevent or delay the development of adverse health outcomes during adulthood and elderly

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