eAppendix 1

To remove extraneous contaminants, each sample was pre-cleaned before analysis by the following procedure: the samples were sonicated for 15 minutes in approximately 10 mL of 1% Triton X-100 solution in 15-mL plastic tubes, rinsed several times with distilled deionized water, dried at 60°C for 24 hours in a drying oven and weighed into a 15-ml plastic tube. The samples were then digested with 1 ml of concentrated HNO_3 acid for 24 hours, diluted to 5-ml with deionized water and analyzed by ICP-MS (Elan 6100, Perkin Elmer, Norwalk, CT) using an external calibration method with tellurium as the internal standard for As.

Quality control (QC) measures included analyzing the initial calibration verification standard [standard reference material 1643e (trace elements in water); National Institute of Standards and Technology Gaithersburg, MD], continuous calibration standards, a solution of 1 ng/ml mixed-element standard As solution (NIST traceable), and a procedural blank. The QC sample was Certified Reference Material GBW 07601. Recovery of the QC standard analysis was 90% -110% with 95% precision.

The inter-assay coefficient of variation was 0.1. The detection limit for the analytical solution was 0.2 ng/mL. Due to sample weight variability (range: 0.002g-0.9g), the samples DL varied from 0.001 μg/g to 0.42 μg/g (mean = 0.02 μg/g) (sample DL= DL for the analytical solution x dilution factor).

eAppendix 2

The structure of the fitted models is described by the following equation:

$$Y_{ijk} = \beta_0 + u_k + \beta_E * \text{Toenail As}_{jk} + \beta_L * \text{CpG locus}_{ijk} + \beta' X_{jk}' + e_{ijk}$$

where Y_{ijk} is the DNA methylation level in either Alu or LINE-1 repetitive element CpG locus i at time j in subject k; β_0 is the overall intercept; u_k is the random intercept for subject k; β_E is the slope representing the fixed effect of toenail As; β_L is the slope representing the fixed effect of the CpG dinucleotide locus; β' is the vector of the regression coefficients for the covariates (vector of X_{jk}) at time j for subject k adjusted in the model; and e_{ijk} is the residual error term.

eAppendix 3

The influence diagnostics included measures of overall influence (i.e. likelihood distance), measures of influence on the estimates of the fixed effects and of the covariance parameters (i.e. Cook's D and the multivariate DFFITS statistic), measures of influence on the precision in the estimates of the fixed effects and of the covariance parameters (i.e. covariance trace and covariance ratio), measures of changes in the effect estimate of interest when removing participants or observations.

eAppendix 4

Wright et al. found a negative association between patella lead and LINE-1 DNA methylation but not Alu.[1] Unfortunately, there was a great amount of missing information on patella lead measurements in our specific study population with only 181 study visits

(from a total of 735) having available patella lead measurements. To evaluate potential confounding by lead, we examined the correlation between toenail arsenic and patella lead. We found that there was no correlation (spearman correlation coefficient (r_s)= -0.07, p-value=0.350, n=181). The half life of bone lead is ≥10 years and thus bone lead is considered a measure of long-term exposure. Therefore, we believed that it would be reasonable to assign this long-term measure of exposure to the visits of the same individual that were relatively close in time in order to increase the number of visits with available bone lead measurements. We assigned the immediate previous (if previous was not available, we assigned the immediate next in time) patella lead measurement to the missing values of patella lead. For those individuals who missed both, the missing value remained missing. After doing that, the number of visits with available patella lead measurements was increased to 429 (from a total of 735) and again no correlation between patella lead and toenail arsenic was detected [r_s= -0.04, p-value=0.618 at baseline visit (n=168) and rs=-0.03, p-value=0.516, for all visits (n=429)]. Since lead exposure was not associated with As exposure, we did not further considered this covariate in our analysis.

A negative association was found between black carbon exposure and Alu DNA methylation as well as between SO_4 and LINE-1 DNA methylation by Madrigano et al. among NAS participants.[2] Specifically, an interquartile range increase in BC over a 45-, 60- and 90-day period was associated with a decrease of 0.17% (95%CI, -0.34 to 0.00), 0.21% (95%CI, -0.39 to -0.03) and 0.31% 5-methylcytosine (95% CI, 0.12-0.50%) in Alu respectively. An interquartile range increase in SO_4 over a 90-day period was associated with a decrease of 0.27% 5mC (0.02-0.52%) in LINE-1. In order to examine the

association between the air pollutants black carbon and SO_4 and our exposure of interest, we calculated the spearman correlation coefficients between toenail arsenic with the 45-, 60-, and 90-day moving average of black carbon as well as the 90-day moving average of SO_4 and the results are presented in eTable 1. Given the lack of association between toenail arsenic and black carbon, we did not consider this covariate further in our analysis.

Since the 90-day moving average for SO_4 was associated with toenail arsenic, we included the covariate in our models for LINE-1 to check whether it will influence our findings. Since there were missing SO_4 measurements, we re-ran our models using only the visits which have data on SO_4 and the results for the main effect of toenail arsenic original model and the model additionally adjusted for SO_4 are presented in eTable 2. The main effect of toenail arsenic on LINE-1 DNA methylation in this restricted population did not change when SO_4 was included in the model.

Also, Baccarelli et al. have also reported a negative association between recent higher black carbon and PM2.5 exposure (7-d moving average) and LINE-1 methylation.[3] We explored whether there was an association between toenail arsenic and both 7-day averages of black carbon and PM2.5 in our study population and we found no correlation between arsenic and black carbon (r_s=-0.00, p-value=0.997, n=735) or arsenic and PM2.5 (r_s=-0.00, p-value=0.953, n=735). No correlations were also found at first visit only.

eTable 1. Spearman correlation coefficients between toenail As and air pollutants among the study population (n=735 visits)

Air Pollutant (average)	Number of visits	Spearman r	p-value
Black carbon			
45 days	735	0.05	0.202
60 days	735	0.02	0.600
90 days	735	-0.02	0.673
SO_4			
90 days	594	0.10	0.014

eTable 2. Estimated changes in LINE-1 DNA methylation associated with one interquartile change (0.06μg/g) in toenail among the study population with complete data on SO_4 (n=1782 CpG methylation observations from 594 visits).

LINE-1	Observations	Beta (95% CI)
Original Model[a]	1782	0.02 (-0.06 to 0.09)
Additionally adjusted for SO_4	1782	0.02 (-0.06 to 0.09)

[a] Multivariable adjusted mixed effects model adjusted for age, laboratory batch, CpG locus number, % lymphocytes, alcohol drinking status, and body mass index.

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