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Supplemental Material

Association of Glyphosate Exposure with Blood DNA Methylation in a Cross-Sectional Study of Postmenopausal Women

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Figure S1. Calibration curve for a) glyphosate and b) AMPA ranging from 0 to 5 ng/mL in urine. The area ratio represents the ratio of variable concentration of glyphosate and AMPA (0 to 5ng/mL) to their respective internal standards ($^{13}\text{C}_2^{15}\text{N}$ -Glyphosate and $\text{D}_2^{13}\text{C}^{15}\text{N}$ -AMPA) with a constant concentration of 6.25 ng/mL spiked in urine. AMPA: aminomethylphosphonic acid.

Figure S2. Histograms of urinary glyphosate (A) and AMPA (B) concentration measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS) in 392 postmenopausal California women, averaged for two samples from each individual, and histograms of the natural log of urinary glyphosate (C) and AMPA (D). LOD: 0.014 ng/mL for glyphosate and 0.013 ng/mL for AMPA. AMPA: aminomethylphosphonic acid.

Figure S3. Scatter plots of between-sample glyphosate (A) and AMPA (B) concentrations measured by LC-MS/MS in postmenopausal California women (n = 388 individuals with paired urine samples). Panel C shows the relationship between glyphosate and AMPA concentration within-samples (n = 780 samples from 392 individuals). All concentrations are transformed with the natural logarithm and divided by the urinary creatinine concentration to account for per-sample differences in urine concentration. AMPA: aminomethylphosphonic acid.

Figure S4. Correlation of epigenetic age from the Hannum (A), Horvath (B), and Levine (C) epigenetic clocks, epigenetic age acceleration from each clock (E-G), and DunedinPoAm (H) compared to chronologic age in 392 postmenopausal California women. Also shown is a histogram of standardized (z-score) DunedinPoAm (D). Epigenetic age acceleration was calculated by forming residuals from a model regressing chronologic age on epigenetic age from each clock. Dotted lines show where chronologic age equals epigenetic age (panels A-C) or where epigenetic age acceleration or epigenetic pace of aging (PoA) equals zero (panels D-H).

Figure S5. Performance of methylation index using 14 CpG sites on the 450K methylation array to predict urinary glyphosate concentration in the training set (A) and the validation set (B, C). Panel D shows the classification performance of the methylation index in the validation set for classifying the highest vs. the lowest tertile of urinary glyphosate. The index was developed using elastic net regression on methylation β values of differentially methylated probes associated with glyphosate and found on the Illumina 450K methylation array in the training set, a population of 332 postmenopausal California women.

Figure S6. Performance of methylation index using 2 CpG sites to predict urinary AMPA concentration in the training set (A) and the validation set (B, C). Panel D shows the classification performance of the methylation index in the validation set for classifying the highest vs. the lowest tertile of urinary AMPA. The index was developed using elastic net regression on methylation β values of differentially methylated probes associated with AMPA in the training set, a population of 332 postmenopausal California women. AMPA: aminomethylphosphonic acid.