

Quantifying Fecal Phthalate Exposure in the Microbiome in Alzheimer’s Risk Study

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Introduction:

- Phthalates are synthetic diesters of phthalic acid, widely used in plastics, consumer goods, and personal care products.
- They dissolve readily in fats and oils, migrating from plastic packaging into fatty foods (oils, dairy, meat, processed snacks).
- They disrupt the gastrointestinal barrier, gut microbial composition, immune function, and endocrine signaling.
- These mechanisms are linked to inflammation, metabolic dysfunction, and neurodegenerative risk across the life course.
- Older adults may be especially vulnerable, as age-related declines in detoxification capacity and blood-brain barrier deterioration may increase susceptibility to phthalate accumulation.
- Microplastics detected in postmortem brain tissue at concentrations 5–8x higher in individuals with dementia
- The pathway connecting **phthalate exposure** → **gut disruption** → **neurological risk** remains largely uncharacterized; human fecal phthalate biomonitoring methods are virtually absent from the literature

Research Questions:

RQ1: Can **fecal phthalate** analysis **methods** be optimized to **reliably detect** phthalates and their metabolites above methodological **sensitivity thresholds**, while minimizing contamination and storage-related bias? (n = 10) **H1:** *Optimized storage, contamination control, and analytical procedures will improve signal validity and detection reliability. Multiple phthalates and metabolites will be detectable above methodological sensitivity thresholds in fecal samples.*

RQ2: What are the gut microbiome **profiles** and microbial metabolite **patterns** among MARS participants undergoing sequencing analysis? (n = 315) **H2:** *Participants will demonstrate variability in microbial diversity, genus-level composition, and microbial metabolite concentrations.*

RQ3: Are **fecal phthalate concentrations** associated with **gut microbiome** composition, **geo-social** factors, and **brain health** measures within the pilot sample? (n = 10) **H3:** *Higher fecal phthalate concentrations will be associated with reduced microbial diversity, higher area deprivation scores, and poorer brain health outcomes.*

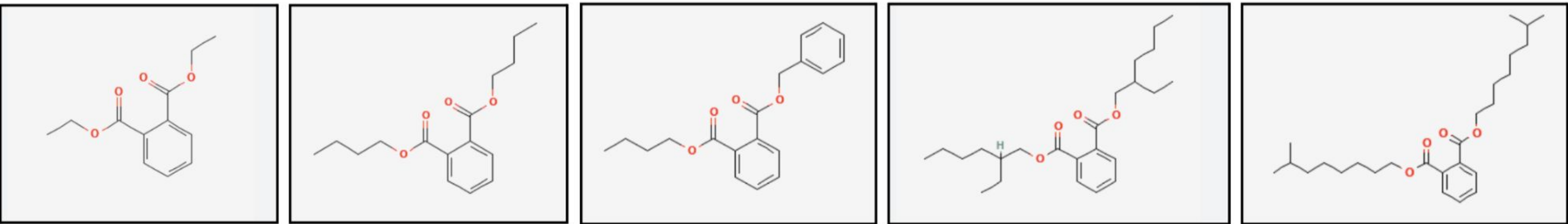


Figure 1. PubChem 2D chemical structural images of parent phthalates examined in this project. From left to right: Di-ethyl phthalate, Di-n-butyl phthalate, Butylbenzyl phthalate, Di-(2-ethylhexyl) phthalate, Di-iso-nonyl phthalate.

Methods

Fecal samples were **collected at home** by participants and **frozen at -80°C**. Sequencing was conducted at the **Rey Lab (UW-Madison)**. Samples were then shipped to **UC-Santa Barbara**, where we adapted urinary extraction protocols for fecal matrices and used **UPLC-MS** (Ultra-High Performance Liquid Chromatography Mass Spectrometry) to separate, identify, and quantify phthalate compounds. A key challenge is the **"phthalate blank" problem** — because phthalates are ubiquitous in lab environments, contamination control is central to our method development.

Data Sources

- Microbiome in Alzheimer's Risk Study (MARS) (2016–present)
- Neighborhood Atlas Area Deprivation Index (ADI)
- Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND diet)

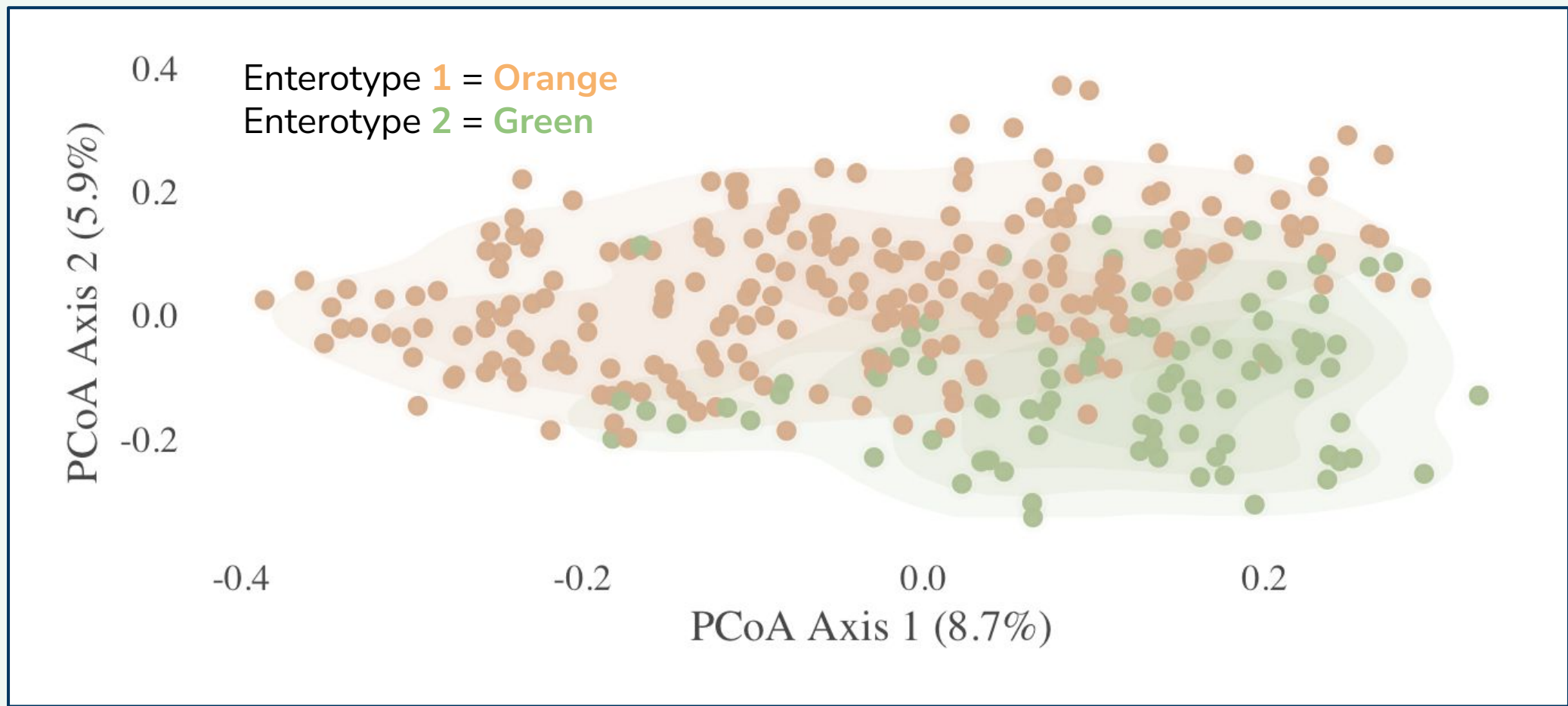
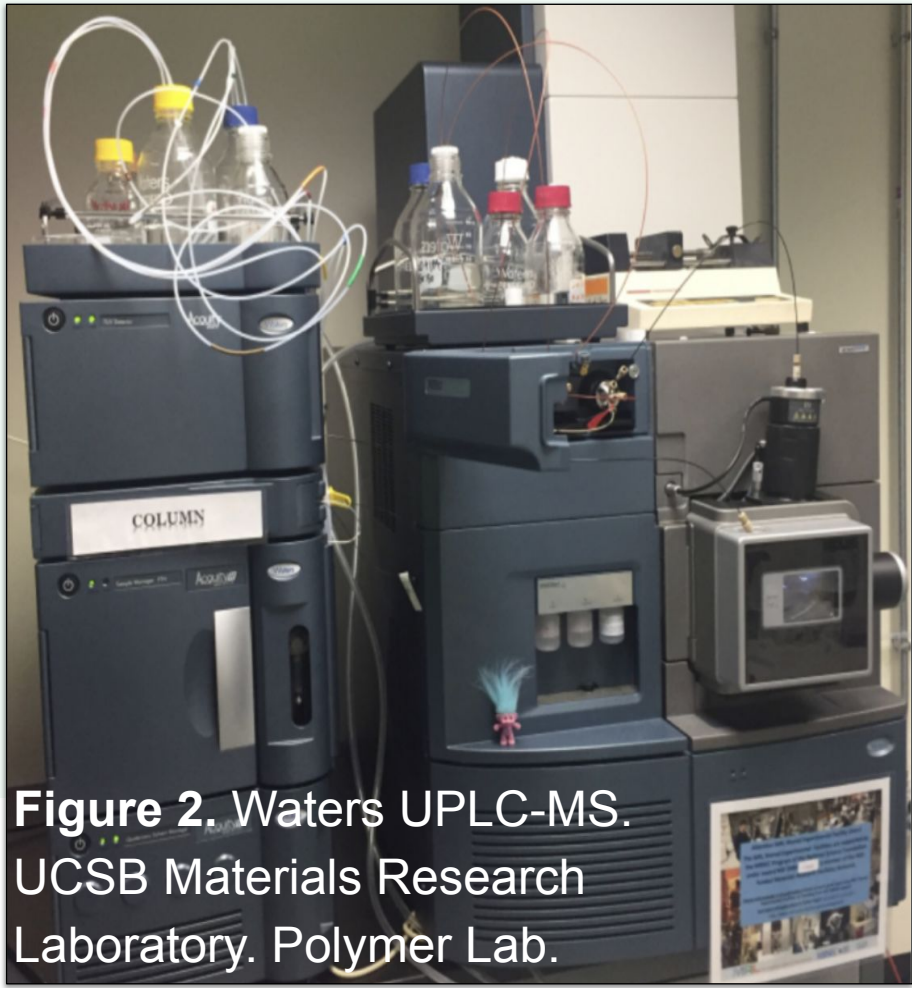


Figure 3. Gut microbiome enterotypes among Microbiome in Alzheimer's Risk Study (MARS) participants (n = 315). Colors denote enterotypes based on dominant genera.

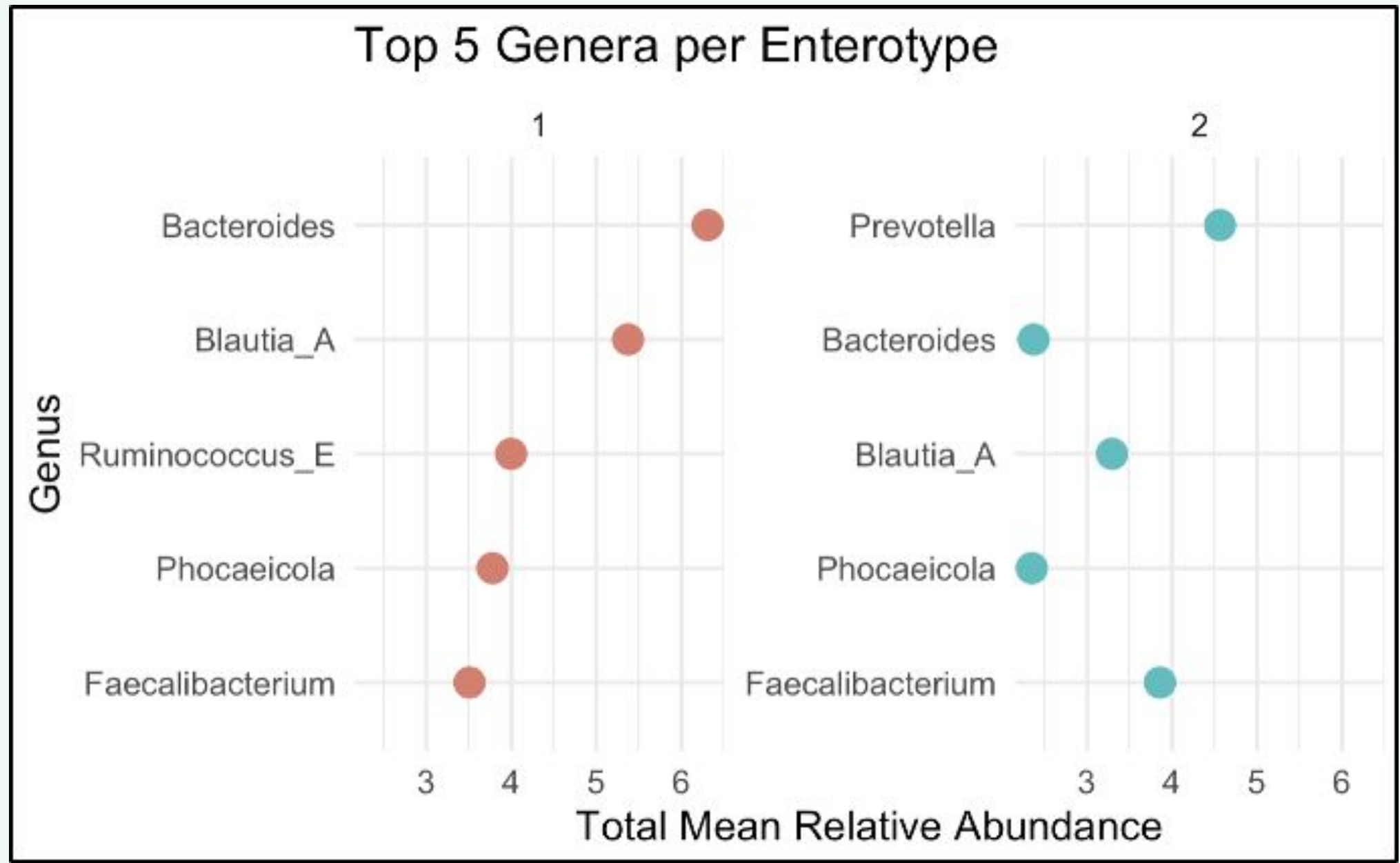


Figure 4. Relative abundance of the top five bacterial species within each enterotype.

Phthalates	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Prevalence across samples
Di-ethyl phthalate											0%
Mono-ethyl phthalate											80%
Di-n-butyl phthalate											0%
Mono-n-butyl phthalate											0%
OH-mono-n-butyl phthalate											20%
Mono-(3-carboxypropyl) phthalate											60%
Butylbenzyl phthalate											100%
Mono-benzyl phthalate											10%
Di-(2-ethylhexyl) phthalate											90%
Mono-(2-ethylhexyl) phthalate											0%
Mono-(2-ethyl-5-hydroxy-hexyl) phthalate											0%
Mono-(2-ethyl-5-oxo-hexyl) phthalate											50%
Mono-(2-ethyl-5-carboxy-pentyl) phthalate											40%
Mono-(2-carboxymethyl-hexyl) phthalate											40%
Di-iso-nonyl phthalate											20%
Mono-isononyl phthalate											60%
Mono-(hydroxy-iso-nonyl) phthalate											30%
Mono-(oxo-iso-nonyl) phthalate											30%
Mono(carboxy-iso-octyl) phthalate											20%
Prevalence within samples	32%	26%	32%	21%	47%	58%	37%	26%	16%	47%	

Figure 5. Detection of 19 phthalates in ten fecal samples from the Microbiome in Alzheimer's Risk Study (MARS (n = 10).. Green circles indicate strong signal, yellow circles indicate trace, and gray circles indicate non-detection.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Sociodemographics										
Age	66	53	76	72	77	64	66	67	70	62
Sex	female	male	female	female	male	female	female	male	female	female
Race	white	white	black or AA	white	white	black or AA	white	white	white	white
Education	13	16	10	16	15	15	15	19	14	16
Geography										
ADI	1	9	10	9	1	1	10	1	1	9
Gut										
Bristol	5	4	4	4	4	3	3	4	3	4
BMI	28.33	34.67	41.79	32.15	26.39	44.72	26.57	23.11	27.89	33.81
MIND	7	4.5	5	9	10	8.5	10	7.5	10.5	6
Enterotype	1	2	1	2	2	1	1	1	1	2
Shannon Index	4.04	3.76	3.54	3.63	4.46	3.62	4.03	4.13	3.87	3.89
Observed Species	175	165	89	142	238	102	176	207	135	198
Brain										
Diagnosis	cog. normal	cog. normal	cog. normal	cog. normal	cog. normal	cog. normal	cog. normal	cog. normal	cog. normal	cog. normal
Plasma GFAP mean conc.	61.03	72.00	158.02	68.93	156.51	78.23	125.82	78.69	82.06	187.44
Plasma NFL	13.11	5.03	9.63	13.14	25.82	15.08	18.47	12.13	11.12	14.91
Plasma ptau 217	0.20	0.21	0.18	0.34	0.23	0.23	0.23	0.50	0.20	0.95
Prevalence within samples	32%	26%	32%	21%	47%	58%	37%	26%	16%	47%

Table 1. Geo-social measures, cognitive biomarkers, and phthalate prevalence in ten fecal samples from the Microbiome in Alzheimer's Risk Study (MARS).

Results & Discussion

RQ1 — Method optimization for fecal phthalate analysis of presence and burden (**H1: SUPPORTED**) Analytical procedures successfully detected phthalates while addressing challenges related to storage conditions, environmental contamination, and signal validity. Two parent phthalates — butylbenzyl phthalate (100% prevalence) and DEHP (90% prevalence) — were detected across nearly all samples, regardless of deprivation status, diet quality, or enterotype.

RQ2 — Gut microbiome composition and enterotypes (**H2: SUPPORTED**) Distinct enterotype profiles emerged, with Enterotype 1 characterized by *Bacteroides* dominance and Enterotype 2 characterized by higher *Prevotella* abundance. Enterotype 2 also demonstrated significantly greater alpha diversity and species richness.

RQ3 — Phthalates, gut microbiome, and brain health relationships (**H3: PARTIALLY SUPPORTED**) Participants with Enterotype 2 tended toward higher ptau-217 values, and Sample 10 — characterized by high phthalate burden, high ADI, and poor brain biomarker profile — may represent a convergence of gut-brain-environmental risk factors, though the pilot sample size limits inference.

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