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Breath Biomarkers to Measure Uptake of Volatile Organic Compounds by Bicyclists

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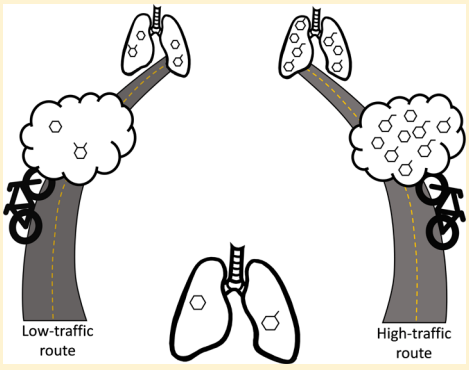
Supporting Information

ABSTRACT: Breath biomarkers were used to study uptake of traffic-related volatile organic compounds (VOCs) from urban bicycling. Breath analysis was selected because it is one of the least invasive methods to assess urban traveler exposure. Research hurdles that were overcome included considering that factors other than on-road exposure can influence concentrations in the body, and absorbed doses during a trip can be small compared to baseline body burdens. Pre-trip, on-road, and post-trip breath concentrations and ambient air concentrations were determined for 26 VOCs for bicyclists traveling on different path types. Statistical analyses of the concentration data identified eight monoaromatic hydrocarbons potentially useful as breath biomarkers to compare differences in body levels brought about by urban travel choices. Breath concentrations of the biomarker compounds were significantly higher than background levels after riding on high-traffic arterial streets and on a path through a high-exposure industrial area, but not after riding on low-traffic local streets or on other off-street paths. Modeled effects of high-traffic streets on ambient concentrations were 100−200% larger than those of low-traffic streets; modeled effects of high-traffic streets on breath concentrations were 40−100% larger than those of low-traffic streets. Similar percentage increases in breath concentrations are expected for bicyclists in other cities.



1. INTRODUCTION

Most bicyclists and other active travelers obtain health benefits from the associated physical activity,[1] but they also risk uptake of traffic-related toxicants.[2−4] Past research has shown that increasing the separation of bicyclists from motor vehicles can reduce exposure,[5−7] but absorbed doses do not always depend in a simple way on exposure. Understanding how exposure concentrations map to absorbed doses can be challenging when ambient concentrations are not constant and the human demographic, the level of physical activity, and the physiologic response to the activity all have wide statistical variance.

Exposure biomarkers have been used as indicators of absorbed dose, and for estimation of the expected consequent risks.[8] Two studies have measured traffic-related exposure biomarkers for bicyclists: Bergamaschi et al.[9] observed significant increases in the levels of benzene, toluene, and xylenes in blood and urine of bicyclists after riding in urban areas, but not after riding in rural areas; using induced sputum samples, Nwokoro et al.[10] concluded that inhaled doses of black carbon particulate matter in London were higher for bicyclists than nonbicyclists. Inhaled doses of particulate matter have also been modeled to compare bicyclists with other travel modes.[11,12] Absorbed dose by path within an urban transportation network has been scarcely studied.

Breath analysis has emerged as a useful method of obtaining volatile organic compound (VOC) exposure biomarker data.[13,14] The assumption usually employed is that toxicant concentrations in end-tidal exhaled air are closely related if not proportional to the corresponding concentrations in blood.[8,15] Breath biomarkers are potentially well-suited to the study of VOC uptake by travelers because breath samples can be collected in situ at a relatively high frequency: less invasively than blood and more frequently than urine.

Application of breath biomarkers to travelers is difficult if absorbed doses of VOCs during a trip are small compared to the baseline body burden of an urban resident. Transportation microenvironments often contain high concentrations of traffic-related VOCs,[16,17] but typical exposure durations can be short and even air in rural locations or urban greenspaces contains detectable levels of many traffic-related VOCs.[18] Another challenge when using breath biomarkers is that a great many compounds are endogenously produced and thus exhaled in normal human breath.[13,19] The high water content in exhaled breath can impede accurate quantification of compounds that are hydrophilic and thus tend to be lost to liquid water that has condensed in the sampling apparatus, or for which water

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interferes during analysis. Airway gas exchange can also affect exhaled breath concentrations for hydrophilic compounds.[20] Last, for breath analysis to be useful in assessing the effect of a travel variable on toxicant uptake, the "signal" of absorbed dose difference must be significantly higher than the "noise" created by background exposure, endogenous production, and other factors that can influence blood and breath concentrations besides on-road exposure.

This paper describes what we believe is the first application of breath analysis to study the uptake of VOCs by travelers. The objectives were to (1) obtain on-road measurements of VOCs in ambient air and in the exhaled breath of urban bicyclists, (2) examine the feasibility of using breath biomarkers to measure differences in absorbed dose by urban path type, and (3) quantify the differences in breath concentrations of VOCs for bicyclists on high-traffic and low-traffic streets.

2. EXPERIMENTAL SECTION

The data set was obtained using a total of three human subjects; this number was considered adequate because the primary focus of the study involved environmental factors, which were assumed to have a larger influence on uptake during travel than between-subject factors.[21] Measurements were carried out in Portland, Oregon, USA over the morning peak travel period (7:00−10:00 am) on 9 days in April through September 2013. On each day and for each rider, a preride period of 30 min within a 0.8 km^2 park (Mt Tabor City Park; 45°30′42″ N, 122°35′44″ W) located 5 km from the city center was used in an effort to bring blood concentrations toward an equilibrium with the urban background. Sampling details are given in the Supporting Information (SI).

Prescribed riding segments during exposure sampling were 6−9 km (requiring 22−38 min to complete) and each comprised a single roadway facility type. Time-averaged ambient VOC concentrations were measured for the full ride time of each segment: ambient air was sampled through stainless steel adsorption/thermal desorption (ATD) cartridges (Tenax TA plus Carbotrap B) as in Pankow et al.[22] Each cartridge was attached to the handlebars at 1.0 m above road level. The pump used was from SKC (Eighty Four, PA), model PCXR8, set at 50 or 75 mL min^{-1} for a total sample volume of ∼2 L on each segment. End-tidal breath samples of 1.5−2.0 L were collected roadside before and after each segment; only the second half of an exhaled breath was collected, to avoid dead-space respiratory air.[23] Each breath sample was acquired using a 3-L FlexFilm (SKC) bag fitted with a mouthpiece. The ATD cartridges and breath sample bags were immediately returned to the laboratory at the end of each ride. Each bag was processed in the laboratory using an ATD cartridge.

Every sample was analyzed on the day collected. Each ATD cartridge was thermally desorbed (TurboMatrix 650 ATD, PerkinElmer, Waltham, MA) and analyzed for VOCs using an Agilent (Santa Clara, CA) 7890A gas chromatograph and 5975C mass spectrometer (details including minimum detectable levels are in Pankow et al.;[24] see also Pankow et al.[18,25]). Sample concentrations were determined for 75 target compounds. Blank corrections were made using travel and lab blanks. Other details are given in SI Table S2.

Three statistical procedures were utilized to identify compounds that can be used for breath biomarkers in transportation microenvironments.

1. Breath concentrations less than ambient concentrations, indicating uptake through inhaled air:

   Concurrent breath and ambient concentrations are compared using the breath/ambient concentration ratio, defined for riding segment i as $\mathrm{BAR}_i = \frac{C_i^{\mathrm{br}}}{C_i^{\mathrm{am}}}$, where C_i^{br} and C_i^{am} are the end-segment breath and in-segment ambient concentrations. Because of a non-normal (positive skew) distribution, BAR_i is compared to 1 using a two-sided Wilcoxon signed rank test; $\mathrm{BAR}_i < 1$ is accepted at $p < 0.05$.
2. Ambient concentrations during travel higher than the urban background:

   A linear mixed effects (LME) model is estimated with the specification

$$\ln(C_i^{\mathrm{am}}) = \alpha_0 + \sum_k \gamma_k L_{k,i} + \sum_j \beta_j X_{j,i} + \rho_m + \varepsilon_i \quad (1)$$

   where i is an observation index, $L_{k,i}$ is a dummy variable for observation i at location k, $X_{j,i}$ is additional covariates (e.g., wind speed, temperature), α_0 is an intercept, γ_k and β_j are estimated fixed effect coefficients, ρ_m is the random effect for samples collected on day m, and ε_i is a random error term. Using the background location (Mt Tabor City Park) as the reference level ($k = 0$) for $L_{k,i}$, elevated ambient concentrations during travel are determined by significant positive γ_k estimates.
3. An association between breath and ambient concentrations:

   A second LME model is estimated with the specification:

$$\ln(C_i^{\mathrm{br}}) = \alpha_0 + \alpha_1 \ln(C_i^{\mathrm{am}}) + \sum_j \beta_j X_{j,i} + \tau_s + \varepsilon_i \quad (2)$$

   where $X_{j,i}$ is a set of covariates (e.g., temperature, heart rate, presegment concentrations), α_0 is an intercept, α_1 and β_j are estimated fixed effect coefficients, τ_s is the random effect for subject s, and ε_i is the random error. An association between breath and ambient concentrations is determined by a significant positive α_1 estimate.

After establishing the biomarker compounds, the third research objective is addressed with a LME model specified:

$$\ln(C_i^{\mathrm{br}}) = \alpha_0 + \sum_k \gamma_k L_{k,i} + \sum_j \beta_j X_{j,i} + \tau_s + \rho_m + \varepsilon_i \quad (3)$$

where all terms are as defined above. Estimates of γ_k indicate the effects of high-traffic and low-traffic locations (k) on breath concentrations. Natural log transformations are used in all of the LME models to address non-normal concentration distributions for C_i^{am} and C_i^{br}. Potential covariates X_j are tested by stepwise addition to each model, retained if the estimated β_j are significant at a 95% confidence level. Tested potential covariates include meteorology variables (wind speed, temperature, humidity), physiology variables (heart rate, breathing rate), segment duration, and lagged dependent (concentration) variables (see SI for data sources). LME models are estimated by maximizing restricted log-likelihood with the "lme4" package in the statistical software R.[26] Estimated models are checked for collinearity of covariates, error distribution, and error correlation (see SI Section 4).

3. RESULTS

The weather, travel, and subject physiology conditions during on-road sampling are summarized in SI Table S1. A total of

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51 ambient samples and 74 breath samples were obtained (some samples were for paired riders on the same route). Of 75 target analytes, the 26 in Table 1 were above the detection limit of 0.05 ng L^{-1} in at least 50% of both on-road ambient and on-road breath samples (see SI Table S3). On the basis of their mass spectra, 43 additional nontarget analytes were tentatively identified in breath air, including sulfur-containing compounds such as dimethyl sulfide and 3-(methylsulfanyl)-1-propene, aldehydes such as acetaldehyde and hexanal, alcohols such as ethanol, 2-propanol, and 2-ethyl-1-hexanol, terpenes such as isoprene, pinene, and limonene, and ethers such as dioxane (see SI Table S4).

Median values of BAR and test results for BAR = 1 are given in the last two columns of Table 1. The results have been interpreted as follows:

> BAR < 1: the compound is primarily of exogenous origin, and is being absorbed from inhaled air
> BAR ≈ 1: the body level of the compound is in some state of equilibrium, with uptake from inhaled air (and possibly endogenous production) balanced by loss through exhalation (and possibly metabolic clearance)
> BAR > 1: the compound is primarily of endogenous origin (or was previously absorbed), and is being cleared from the body by exhalation (and possibly metabolic clearance).

The data for 10 aromatic hydrocarbons and 3 halocarbons exhibit a primarily exogenous characteristic (BAR < 1).

Sampling locations were separated into four categories, with breath observations of 21, 8, 23, and 22, respectively: "background" (Mt Tabor City Park), "off-street" (off-street bicycle and pedestrian paths), "local street" (traffic-calmed local streets with average daily traffic (ADT) under 2000),[27] and "arterial street" (collector and arterial streets with ADT over 2000). Mean ADT on local streets and arterial streets was 1000 and 19 800 vehicles day^{-1}, respectively. Preliminary analysis revealed a "hot-spot" high exposure location on an off-street path through an industrial area (see SI) with ambient BTEX (benzene, toluene, ethylbenzene, and xylenes) concentrations higher than all other off-street paths by more than a factor of 2. To separate this hot-spot location, off-street paths are divided into "off-street/industrial" and "off-street/other".

Table 2 gives eq 1 model estimation results for the 13 compounds with BAR < 1. Ambient concentrations are significantly higher than the background in three of the four travel locations for the aromatics, but not the halocarbons. For the aromatics, γ_4 and γ_2 are not significantly different from each other and both are significantly higher than γ_3 and γ_1. The aromatics are negatively influenced by wind speed, as expected, and the lagged dependent variable is significant and positive for 6 of the aromatics.

Table 1. Characterization of VOC Concentrations in Breath and Ambient Air Sampled On-Road (ng L^{-1})

	ambient			breath			BAR	
	median	mean	range[a]	median	mean	range	median	test[b] of BAR = 1
Halocarbons								
trichlorofluoromethane (CFC11)	0.69	0.72	(0.45−1.09)	0.61	0.65	(0.42−1.27)	0.91	reject: lower
methylene chloride	0.65	0.79	(0.27−3.49)	0.58	1.22	(ND−7.24)	0.85	accept
1,1,2,-trichloro-1,2,2-trifluoroethane (CFC113)	0.60	0.61	(0.50−0.75)	0.53	0.53	(0.45−0.70)	0.88	reject: lower
chloroform	0.13	0.14	(0.07−0.48)	0.16	0.17	(0.08−0.36)	1.3	reject: higher
carbon tetrachloride	0.51	0.51	(0.44−0.64)	0.38	0.39	(0.31−0.48)	0.77	reject: lower
tetrachloroethene (PCE)	0.32	0.37	(0.07−1.24)	0.29	0.36	(0.10−1.79)	1.3	reject: higher
Esters								
methyl acetate	0.12	0.13	(ND−0.40)	7.12	7.09	(1.91−15.02)	54	reject: higher
methyl methacrylate	0.16	0.25	(ND−3.79)	0.78	0.93	(0.30−5.34)	5.2	reject: higher
Sulfides								
carbon disulfide	0.05	0.08	(ND−0.53)	1.31	1.70	(0.59−11.58)	30	reject: higher
Ketones								
2-butanone	0.80	1.05	(0.53−3.33)	2.34	2.43	(1.24−4.30)	2.5	reject: higher
acetone	4.52	4.82	(1.46−13.40)	388.5	412.7	(220.1−814.2)	90.5	reject: higher
4-methyl-2-pentanone (MIBK)	0.10	0.11	(ND−0.39)	0.12	0.11	(ND−0.32)	0.91	accept
2-hexanone (MBK)	0.06	0.06	(ND−0.17)	0.08	0.07	(ND−0.20)	1	reject: higher
Aromatics								
benzene	1.35	1.67	(0.19−7.43)	0.67	0.87	(0.16−3.97)	0.50	reject: lower
toluene	3.20	4.03	(0.73−16.91)	1.20	1.38	(0.46−3.58)	0.35	reject: lower
ethylbenzene	0.71	0.85	(0.19−2.86)	0.16	0.19	(0.08−0.42)	0.23	reject: lower
m- + *p*-xylene	2.61	3.16	(0.71−10.35)	0.53	0.62	(0.28−1.46)	0.19	reject: lower
ethenylbenzene (styrene)	0.21	1.44	(ND−32.30)	0.17	0.21	(0.07−0.92)	0.60	accept
o-xylene	0.93	1.14	(0.27−3.78)	0.18	0.22	(0.12−0.51)	0.19	reject: lower
n-propylbenzene	0.18	0.21	(0.06−0.71)	0.09	0.10	(0.05−0.25)	0.5	reject: lower
1,3,5-trimethylbenzene	0.24	0.30	(0.08−1.04)	0.07	0.07	(ND−0.17)	0.2	reject: lower
2-ethyltoluene	0.21	0.26	(0.07−0.94)	0.05	0.06	(ND−0.12)	0.2	reject: lower
1,2,4-trimethylbenzene	0.78	0.98	(0.26−3.49)	0.15	0.18	(0.08−0.40)	0.20	reject: lower
1-isopropyl-4-methylbenzene	0.14	0.16	(ND−0.38)	0.38	0.49	(0.15−1.78)	2.7	reject: higher
1,2,3-trimethylbenzene	0.21	0.25	(0.07−0.98)	0.05	0.06	(ND−0.17)	0.3	reject: lower
naphthalene	0.26	0.31	(0.06−1.18)	0.21	0.25	(ND−0.92)	0.79	accept

[a]ND = not detected. [b]Two-sided Wilcoxon signed rank test with null hypothesis BAR = 1, rejected at $p < 0.05$.

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Table 2. Models of Ambient Concentration by Location (Eq 1)

	intercept (α_0)	location: off-street/other (γ_1)	location: off-street/industrial (γ_2)	location: local street (γ_3)	location: arterial street (γ_4)	wind speed[b] (β_1)	lagged[c] ambient concentration (β_2)
CFC11	−0.317[a]	0.055	0.119	−0.048	−0.048	0.038	0.075
CFC113	−0.652[a]	−0.057	0.113[a]	0.022	0.038	0.025	−0.194
carbon tetrachloride	−0.563[a]	−0.052	0.024	−0.032	−0.001	−0.009	0.136
benzene	−0.253	0.347	1.117[a]	0.444[a]	1.107[a]	−0.357[a]	0.172[a]
toluene	0.766[a]	0.182	0.957[a]	0.245[a]	0.727[a]	−0.485[a]	0.202[a]
ethylbenzene	−0.683[a]	0.092	1.080[a]	0.431[a]	0.917[a]	−0.384[a]	0.140
m + *p*-xylene	0.446[a]	0.108	1.117[a]	0.456[a]	0.931[a]	−0.394[a]	0.127
o-xylene	−0.424[a]	0.089	1.083[a]	0.451[a]	0.909[a]	−0.397[a]	0.136
n-propylbenzene	−1.842[a]	0.178	1.140[a]	0.486[a]	0.941[a]	−0.327[a]	0.193[a]
1,3,5-trimethylbenzene	−1.832[a]	0.350	1.450[a]	0.664[a]	1.215[a]	−0.360[a]	0.153
2-ethyltoluene	−1.801[a]	0.270	1.261[a]	0.571[a]	1.100[a]	−0.367[a]	0.183[a]
1,2,4-trimethylbenzene	−0.823[a]	0.358	1.444[a]	0.664[a]	1.222[a]	−0.375[a]	0.173[a]
1,2,3-trimethylbenzene	−1.839[a]	0.282	1.310[a]	0.562[a]	1.106[a]	−0.402[a]	0.170[a]

[a]Significant at 95% confidence level. [b]Natural log-transformed wind speed (5 min scalar mean, in m/s). [c]Natural log-transformed presegment ambient concentration.

Table 3. Models of Breath Concentration as a Function of Ambient Concentration (Eq 2)

	intercept (α_0)	ambient concentration (α_1)	lagged breath concentration[b] (β_3)
CFC11	−0.290[a]	0.415[a]	0.102
CFC113	−0.531[a]	0.238[a]	−0.040
carbon tetrachloride	−0.724[a]	0.270[a]	0.037
benzene	−0.161[a]	0.294[a]	0.589[a]
toluene	−0.287[a]	0.324[a]	0.532[a]
ethylbenzene	−0.813[a]	0.272[a]	0.481[a]
m- + *p*-xylene	−0.634[a]	0.310[a]	0.438[a]
o-xylene	−0.861[a]	0.252[a]	0.451[a]
n-propylbenzene	−0.545[a]	0.097	0.702[a]
1,3,5-trimethylbenzene	−1.236[a]	0.170[a]	0.451[a]
2-ethyltoluene	−1.467[a]	0.144	0.414[a]
1,2,4-trimethylbenzene	−1.011[a]	0.149[a]	0.406[a]
1,2,3-trimethylbenzene	−1.268[a]	0.193[a]	0.440[a]

[a]Significant at 95% confidence level. [b]Natural log-transformed presegment breath concentration.

Table 3 gives eq 2 model estimation results for the same 13 compounds; all concentrations are in ng L^{-1}. Breath concentrations are significantly associated with ambient concentrations for 11 of the compounds. The lagged dependent variable is the only other significant covariate. An interaction between subject *s* and ambient concentration ($s \times \ln(C_i^{am})$) was tested and found to be not significant at 95% confidence, indicating minimal between-subject differences in breath−ambient concentration relationships.

Eight biomarkers meet all three criteria (BAR < 1, $\gamma_k > \gamma_0$, and $\alpha_1 > 0$ at $p < 0.05$ in Tables 1−3): the BTEX group and three related monoaromatic compounds. Table 4 gives eq 3 model estimation results for these eight compounds individually and cumulatively and for the five BTEX compounds. The lagged dependent variable is again the only significant covariate (wind speed, temperature, humidity, heart rate, breathing rate, segment duration, and lagged ambient concentration were also tested). For the summed biomarkers, the estimated variance for the subject random effects, date random effects, and residuals are 0.003, 0.077, and 0.043, respectively, indicating the relative importance of between-day variability (likely due to background concentrations and meteorology). The date random effects are significant at a 95% confidence level based on a likelihood ratio test, but the subject random effects are not.

Breath concentrations are only significantly higher than background levels after travel on arterial streets and the industrial hot-spot path; γ_4 and γ_2 for BTEX in Table 4 can be interpreted[28] as expected breath concentrations 24% and 110% higher than the background, respectively. The γ_1, γ_3, and γ_4 estimates are not significantly different from each other at 95% confidence, but γ_2 is significantly higher than the other γ_k. The γ_k coefficient ordering in Table 4 is the same as for ambient concentrations in Table 2 (background < off-street/other < local street < arterial street < off-street/industrial), but the breath concentration differences are only statistically significant for the most extreme comparisons. Compared to background levels, location fixed effects are 100−200% higher for riding on high-traffic arterial streets vs low-traffic local streets (i.e., γ_4 vs γ_3) for ambient BTEX concentrations (Table 2), and 40%−100% higher for breath BTEX concentrations (Table 4).

4. DISCUSSION

Statistical analyses of the data for the on-road samples suggest BTEX and three other related compounds (1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene, and 1,2,3-trimethylbenzene) can be used for breath biomarkers of exposure in transportation microenvironments. All of these compounds are known to be present in exhaust emissions from motor vehicles,[29] and to have some level of toxicity in humans.[30] The BAR values for benzene and toluene in Table 1 are similar to ratios reported in several studies of longer exposure duration and higher exposure concentration.[21,31−33] Wallace et al.[34,35] report lower equilibrium breath/ambient concentration ratios for exposure in a laboratory, but with similar ordering among compounds (benzene > toluene > ethylbenzene ∼ xylenes). The α_1 coefficient of 0.3 for benzene in Table 3 is very similar to previous research that measured changes in benzene in the breath of fuel maintenance workers.[36]

Changes in breath concentrations of the biomarker compounds during riding were smaller than changes in ambient concentrations, which is expected for VOCs with low solubility in blood because uptake is limited by the blood/air equilibrium condition.[34,37] Uptake and biomarker levels can also be reduced by variance in exposure.[38] Breath concentrations after riding on

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Table 4. Models of Breath Concentration by Location (Eq 3)

		location				
	intercept (α_0)	off-street/other (γ_1)	off-street/industrial (γ_2)	local street (γ_3)	arterial street (γ_4)	lagged breath concentration[b] (β_3)
benzene	−0.273	0.270	0.742[a]	0.128	0.253[a]	0.403[a]
toluene	0.031	0.083	0.765[a]	0.140	0.199[a]	0.417[a]
ethylbenzene	−1.078[a]	0.019	0.787[a]	0.151	0.216[a]	0.435[a]
m + *p*-xylene	−0.467[a]	−0.002	0.867[a]	0.141	0.234[a]	0.387[a]
o-xylene	−1.081[a]	−0.001	0.763[a]	0.108	0.151	0.364[a]
1,3,5-trimethylbenzene	−2.020[a]	0.092	0.734[a]	0.149	0.209[a]	0.299[a]
1,2,4-trimethylbenzene	−1.343[a]	0.040	0.787[a]	0.054	0.121	0.289[a]
1,2,3-trimethylbenzene	−2.753[a]	−0.019	0.637[a]	0.052	0.168	0.043[a]
all 8 biomarkers	0.643[a]	0.080	0.768[a]	0.133	0.216[a]	0.387[a]
BTEX	0.575[a]	0.086	0.774[a]	0.140	0.222[a]	0.392[a]

[a]Significant at 95% confidence level. [b]Natural log-transformed presegment breath concentration.

local streets and off-street paths (other than through the industrial hot-spot) were not statistically significantly higher than background levels (Table 4). The challenges of measuring incremental absorbed doses during travel in this study will likely be equally problematic in cities with higher exposure during travel because the associated urban background, residential, and work exposures will also be higher. In a city with exposure levels an order of magnitude higher than this study, BTEX in sampled blood increased 20−40% during urban bicycling (similar to the arterial street fixed effects in Table 4), only statistically significant for benzene and toluene.[9]

Increases in breath concentrations from the urban background are larger riding on high-traffic arterial streets as compared to low-traffic local streets. Ambient concentration differences (Table 2) are consistent with past studies of bicyclist VOC exposure on high-traffic vs low-traffic routes.[4] The BTEX on-road ambient concentrations are similar to recently reported values in Canada,[39] and at the low end of the range of values in previous on-road studies.[4,40−44] The BTEX ambient concentrations at the urban background location (see SI Table S5) are similar to previously measured concentrations in the USA and Canada.[18,45] For bicyclists in any city, it is expected that changes in breath concentrations during riding will be proportional to the differences between on-road and pre-ride exposures, and that uptake of VOCs can be correspondingly reduced by choosing low-traffic bicycle routes.

Many other VOCs besides the eight aromatics identified in this study are present in motor vehicle exhaust and likely taken up by travelers, but are difficult to use as breath biomarkers of exposure. Aromatics are often concentrated near roadways,[46,47] whereas alkanes tend to be more widely distributed due to nonroadway sources,[48] and traffic-related aldehydes such as acetaldehyde and acrolein have large secondary components from oxidation of primary VOC emissions;[49] acetaldehyde was poorly correlated with benzene in the ambient air sample data (see SI Table S4). Breath concentrations of alcohols, acetates, and ketones may be less representative of their respective blood concentrations than breath concentrations of BTEX and other aromatics due to greater solubility in water.[50−53] Some compounds have very low solubility in blood (e.g., short-chain alkanes), and so absorbed doses during riding might be too small to measurably change breath concentrations.[53] Endogenous production is a complicating factor for many compounds; concentrations of methyl acetate and ketones such as acetone, 2-hexanone, and 2-butanone were higher in exhaled breath than ambient air, in agreement with previous studies.[54,55] Even if exposure levels are high enough that a compound is absorbed through inhaled air (BAR < 1), the simultaneous contribution of biological sources to changing blood concentrations can obscure the influence of absorbed dose (likely the case for naphthalene, which has both traffic and biological sources[19]).

Aromatic VOCs have short residence times in the blood,[34] so the 30-min pre-travel equilibration period at Mt Tabor City Park is expected to have largely cleared any previously absorbed doses (above the urban background). However, clearance from less perfused body tissue can take much longer and may have caused some of the observed statistical variance in the breath concentration data. Physiological responses to physical activity levels may also have contributed to the statistical variance,[32,37,56] although the tested physiological covariates for breath concentrations in eqs 2 and 3 were not statistically significant. Physical activity can decrease average alveolar air concentrations through an increase in the ventilation−perfusion ratio,[20,32,57] but the effect on breath concentrations in this study would be negligible because sampled breath air had ample time to equilibrate with blood concentrations (breath samples were collected from a slow expiration with the rider stopped).

A limitation of this study is the number of subjects. The assumption that environmental (between-route) factors have a larger influence on uptake during travel than between-subject factors was supported by the findings that α_1 coefficients in eq 2 relating breath to ambient concentrations were not significantly different among the three (healthy) subjects, and that subject random effects τ_s in eq 3 were not significant. In addition, a previous study of 81 automobile mechanics found that "differences among individuals related to physiological and metabolic characteristics had little influence on benzene uptake."[21] Future work should consider on-road uptake by a broader population of travelers.

Despite the challenges discussed above, we conclude that several aromatic hydrocarbons are potentially useful as breath biomarkers to compare differences in absorbed doses brought about by urban travel choices. Use of breath biomarkers to assess traveler uptake of VOCs should consider (1) the heterogeneity of exposure concentrations along a sampling route or segment, (2) the appropriate breath sampling frequency for a given exposure heterogeneity and physical activity level, (3) the ratio of on-road to urban background ambient concentrations of VOCs in different cities, and (4) the required presampling time to equilibrate blood concentrations for travelers originating from different environments (e.g., home, workplace, restaurant). Additionally, because uptake of particulate matter is fundamentally different from uptake of gases, biomarkers of particulate exposure should also be employed to study absorbed dose differences by urban path type.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b01159.

Details of the on-road sampling procedure and gas analysis conditions, supplemental compound detection and concentration data, and correlations of concentrations among compounds (PDF)

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Notes

The authors declare no competing financial interest.

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