

SUPPLEMENTARY TABLE S1: Precision and accuracy for 1,3-butadiene metabolites in urine,
Inter-day (N=10)

Analyte	Added amount (ng/mL)	Expected amount ^a (ng/mL)	Measured mean (ng/mL)	Accuracy (percent of expected)	Precision CV (%)
Sum of MHBMA-1 and MHBMA-2 (MHBMA1+2)	0		0.11		
	0.5	0.610	0.481	78.9	11.2
	1.0	1.11	0.887	80.0	11.3
	5.0	5.11	4.07	79.6	7.73
	10	10.11	8.17	80.8	7.93
LOQ	0.1				
MHBMA-3	0		0.035		
	1.0	1.04	1.04	100	8.53
	2.5	2.54	2.42	95.5	4.82
	10	10.0	9.55	95.2	4.84
	20	20.0	17.7	88.3	5.16
LOQ	0.1				

^aAdded amount + mean amount measured in non-smoker's urine

MHBMA-1+2 = sum of isomers 1-hydroxy-3-buten-2-yl-mercapturic acid and 2-hydroxy-3-buten-1-yl-mercapturic acid; MHBMA-3 = 4-hydroxy-2-buten-1-yl-mercapturic acid

LOQ = limit of quantitation

SUPPLEMENTARY TABLE S2: Concentrations of urinary biomarkers for Black and White smokers. Biomarkers are presented as raw concentrations, covariate-adjusted standardization of urinary biomarker concentrations, and creatinine-normalized concentrations. The study included a subset of Black and White smokers over the age of 40 who participated in a randomized clinical trial of reduced nicotine content cigarettes between June 2013 and July 2014 at 10 sites across the U.S.

Biomarker	Blacks (GM, 95% CI)	Whites (GM, 95% CI)	p value
TNE (nmol/mL)	40.8 (36.2 - 46.0)	43.2 (38.2 - 49.0)	0.301
TNE (nmol/mL) (covariate-adjusted) †	40.6 (36.7 - 45.0)	43.3 (38.7 - 48.5)	0.152
TNE (nmol/mg) (creatinine-normalized)	40.6 (36.6 - 45.0)	56.9 (50.7 - 63.8)	<0.001
NNAL (pmol/mL)	1.10 (0.96 - 1.27)	1.45 (1.28 - 1.65)	0.005
NNAL (pmol/mL) (covariate-adjusted) †	1.09 (0.97 - 1.23)	1.45 (1.30 - 1.61)	<0.001
NNAL (pmol/mg) (creatinine-normalized)	1.09 (0.97 - 1.24)	1.91 (1.72 - 2.13)	<0.001
2-HPMA (ng/mL)	62.1 (54.5 - 70.7)	62.6 (53.5 - 73.4)	0.756
2-HPMA (ng/mL) (covariate-adjusted) †	62.1 (56.0 - 68.9)	62.6 (55.8 - 70.3)	0.832
2-HPMA (ng/mg) (creatinine-normalized)	62.0 (55.9 - 68.8)	82.3 (73.3 - 92.5)	<0.001
3-HPMA (ng/mL)	1007 (869 - 1166)	965 (834 - 1116)	0.882
3-HPMA (ng/mL) (covariate-adjusted) †	1007 (907 - 1117)	965 (864 - 1077)	0.709
3-HPMA (ng/mg) (creatinine-normalized)	1005 (904 - 1119)	1268 (1136 - 1416)	0.001
AAMA (ng/mL)	205 (181 - 232)	200 (175 - 229)	0.834
AAMA (ng/mL) (covariate-adjusted) †	205 (190 - 220)	200 (185 - 217)	0.856
AAMA (ng/mg) (creatinine-normalized)	204 (190 - 220)	263 (243 - 285)	<0.001
CNEMA (ng/mL)	182 (158 - 210)	184 (159 - 212)	0.377
CNEMA (ng/mL) (covariate-adjusted) †	182 (164 - 202)	184 (163 - 206)	0.281
CNEMA (ng/mg) (creatinine-normalized)	182 (164 - 202)	241 (214 - 272)	<0.001
HEMA (ng/mL)	3.67 (3.09 - 4.36)	3.46 (2.94 - 4.06)	0.739
HEMA (ng/mL) (covariate-adjusted) †	3.67 (3.18 - 4.24)	3.46 (3.02 - 3.95)	0.732
HEMA (ng/mg) (creatinine-normalized)	3.67 (3.17 - 4.24)	4.54 (3.97 - 5.20)	0.015
HPMMA (ng/mL)	209 (160 - 274)	201 (156 - 260)	0.556
HPMMA (ng/mL) (covariate-adjusted) †	209 (162 - 270)	201 (159 - 256)	0.402
HPMMA (ng/mg) (creatinine-normalized)	209 (162 - 269)	265 (209 - 336)	0.013
MHBMA-1+2 (ng/mL)	1.23 (1.03 - 1.46)	1.20 (0.99 - 1.46)	0.603
MHBMA-1+2 (ng/mL) (covariate-adjusted) †	1.23 (1.05 - 1.44)	1.20 (1.01 - 1.44)	0.619
MHBMA-1+2 (ng/mg) (creatinine-normalized)	1.23 (1.05 - 1.43)	1.58 (1.32 - 1.89)	0.004
MHBMA-3 (ng/mL)	0.12 (0.11 - 0.14)	0.14 (0.13 - 0.15)	0.056
MHBMA-3 (ng/mL) (covariate-adjusted) †	0.12 (0.11 - 0.14)	0.14 (0.12 - 0.16)	0.082
MHBMA-3 (ng/mg) (creatinine-normalized)	0.12 (0.11 - 0.14)	0.18 (0.16 - 0.21)	<0.001
MMA (ng/mL)	19.8 (16.6 - 23.6)	14.9 (12.9 - 17.3)	0.023
MMA (ng/mL) (covariate-adjusted) †	19.8 (17.0 - 23.1)	14.9 (13.3 - 16.7)	0.003
MMA (ng/mg) (creatinine-normalized)	19.8 (16.9 - 23.2)	19.6 (17.5 - 22.0)	0.954
PMA (ng/mL)	1.16 (1.00 - 1.35)	1.25 (1.06 - 1.49)	0.242
PMA (ng/mL) (covariate-adjusted) †	1.16 (1.02 - 1.32)	1.25 (1.08 - 1.46)	0.257
PMA (ng/mg) (creatinine-normalized)	1.16 (1.02 - 1.32)	1.65 (1.41 - 1.92)	<0.001

TNE = total nicotine equivalents (nmol/mL); NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (pmol/mL); units of VOC metabolites are ng/mL; 2-HPMA = 2-hydroxypropylmercapturic acid (propylene oxide); 3-HPMA = 3-hydroxypropylmercapturic acid (acrolein); AAMA = 2-

carbamoylethylmercapturic acid (acrylamide); CNEMA = 2-cyanoethylmercapturic acid (acrylonitrile); HEMA = 2-hydroxyethylmercapturic acid (acrylonitrile, vinyl chloride, ethylene oxide); HPMMA = 3-hydroxy-1-methyl-propylmercapturic acid (crotonaldehyde); MHBMA-1+2 = sum of isomers 1-hydroxy-3-buten-2-yl-mercapturic acid and 2-hydroxy-3-buten-1-yl-mercapturic acid (1,3-butadiene); MHBMA-3 = 4-hydroxy-2-buten-1-yl-mercapturic acid (1,3-butadiene); MMA = methylmercapturic acid (methylating agents); and, PMA = phenylmercapturic acid (benzene)

† The covariate-adjusted concentrations were determined using a method from a previously published paper (“Method 3”).(1)

REFERENCES

1. O’Brien KM, Upson K, Cook NR, Weinberg CR. Environmental chemicals in urine and blood: improving methods for creatinine and lipid adjustment. *Environ Health Perspect.* 2016;124:220-7.