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Urinary metabolites of 1-nitropyrene in US–Mexico border residents who frequently cross the San Ysidro Port of Entry

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Abstract

Diesel exhaust presents a community exposure hazard, but methods to measure internal exposure are lacking. We report results from a community-based study using 1-nitropyrene (1-NP) and its urinary metabolites as markers of exposure to traffic-related diesel particulate matter (DPM). The study participants were Tijuana, Mexico residents who commuted on foot into San Diego, California for work or school using the International San Ysidro Port of Entry, placing them within feet of idling traffic (referred to as border commuters). The comparison group (non-border commuters) was comprised of residents of south San Diego who did not commute into Mexico. Air concentration of 1-NP in fine particulate matter ($PM_{2.5}$) was measured in personal samples from participants. Spot urine samples were analyzed for 1-NP urinary metabolites 8-hydroxy-1-nitropyrene (8-OHNP) and 8-hydroxy-*N*-acetyl-1-aminopyrene (8-OHNAAP). Compared with non-border commuters, border commuters had two- to threefold higher mean urinary concentrations for unadjusted and creatinine-adjusted 8-OHNP and 8-OHNAAP. Urinary 8-OHNAAP and the sum of 8-OHNP and 8-OHNAAP were both associated with personal exposure to 1-NP in the prior 24 h. These results suggest that 1-NP urinary metabolites reflect recent exposure to DPM-derived 1-NP in community settings and can be useful for exposure analysis.

Keywords

air pollution; biomarkers of exposure; diesel exhaust; particulate matter; US–Mexico border; 1-nitropyrene

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

INTRODUCTION

Diesel exhaust (DE) is a complex mixture that includes both gas and particle phase components.[1] Epidemiologic and toxicologic studies have demonstrated that exposure to ambient DE is associated with acute and chronic respiratory and cardiovascular adverse health effects and lung cancer. DE has been classified as carcinogenic to humans (group 1).[2] The chemical substances in DE that have been found to contribute to its carcinogenicity and mutagenicity include nitropolycyclic aromatic hydrocarbons (NPAHs).[1] NPAHs are a group of organic compounds that originate directly from incomplete combustion such as from DE emissions, or are formed indirectly as a result of heterogeneous or gas-phase reactions of their parent compounds, PAHs, with atmospheric oxidants such as NO_x and OH.[3,4] NPAHs are often more toxic than their parent PAH compounds.[5]

The compound 1-nitropyrene (1-NP) is a four-ring NPAH that exists primarily in the particulate phase at ambient temperatures,[6] and is one of the main contributors to the direct-acting mutagenicity of NPAHs.[7–9] 1-NP is mostly emitted directly from combustion processes, although small amounts may be formed from secondary reactions.[10] Diesel emissions are the major source of 1-NP, with small amounts emitted from other sources, including coal combustion, soy bean cooking oil, and gasoline vehicles.[11,12] As 1-NP is greatly enriched in diesel emissions compared with other combustion sources, 1-NP in air has been used as a marker of exposure to diesel particulate matter (DPM).[13–15]

Urinary metabolites specific to 1-NP may be useful as biomarkers of exposure to DE. Metabolism of 1-NP has been studied *in vitro* in human and animal cell lines and *in vivo* in rats. Metabolism is reported to proceed via *P*450-mediated C-oxidation, acetylation, and nitroreduction.[16–21] The major urinary metabolites that have been observed in rats and humans *in vivo* are hydroxy-1-nitropyrenes (OHNPs), hydroxy-*N*-acetyl-1-aminopyrenes (OHNAAPs), *N*-acetyl-1-aminopyrene, and 1-aminopyrene.[22–27] Human studies have demonstrated that urinary metabolites of 1-NP are higher in participants with exposure to elevated levels of DPM.[14,28–33] In studies that examined multiple urinary 1-NP metabolites, the most abundant isomers detected were 6- and 8-hydroxy-1-nitropyrene (8-OHNAAP) and 6- and 8-OHNP.[28,31]

Although previous studies have assessed the utility of 1-NP and its metabolites as markers for occupational exposure to DPM,[14,30,31] there are no community-based studies that have measured both personal exposure to 1-NP and urinary 1-NP metabolites. This study addresses the ability of these biomarkers to detect exposures to ambient concentrations of DE in a group of persons residing in Mexico and crossing the US–Mexico border northbound in the pedestrian lane at the San Ysidro Port of Entry (SYPOE), next to long lines of idling diesel buses. Urinary biomarker levels were compared with a control group not crossing the US–Mexico border.

MATERIALS AND METHODS

Study Population

The study population was described in detail in our prior publication.[34] In brief, two groups were recruited for participation in this study. One group ("border commuters") had potentially high exposure to DE as a result of standing 20–200 min within feet of idling buses during their northbound pedestrian commute across the US–Mexico SYPOE. Border commuters lived in Tijuana, Mexico and crossed at the SYPOE on foot to work or go to school in San Diego, California. We also recruited a comparison group ("non-border commuters") that we anticipated to have lower DE exposure. This group included participants who lived and worked or went to school in San Diego and did not cross into Mexico. All participants were self-reportedly free of any chronic lung, liver, and heart disease; all self-classified as Hispanic/Latino, non-smokers in a non-smoking home, and not occupationally exposed to DE. One participant was excluded from analysis as a result of being occupational exposed to DE. Sampling occurred between 30 March 2010 and 17 December 2010. Approval was obtained by San Diego State University and University of Washington institutional review boards for all human participant data collection procedures. Further recruitment details, exclusion criteria, and descriptive statistics are described in detail elsewhere.[34]

Sample Collection

Forty-four participants were enrolled in the study (27 border commuters and 17 non-border commuters). Of the 44 participants, 28 (12 border commuters and 16 non-border commuters) were sampled one time and 16 (15 border commuters and 1 non-border commuter) were sampled more than once with the criterion that 3 weeks had passed since their last participation. As a result of repeat participation, there were a total of 73 border commuter events and 18 non-border commuter events (Table 1). Samples collected per event included a 24-h time activity diary, a questionnaire, a spot urine sample, and, on some participants, a personal air sample for 24 h to measure personal exposure to 1-NP. Urine samples were collected from participants immediately following the end of their 24-h study period. Participants were anticipated to have steady-state concentrations for urinary metabolites owing to consistent daily weekday routines; therefore, concentrations collected were assumed to be representative of their daily values. Urine was not successfully collected from five participants (all border commuters).

1-NP air concentrations were measured on a subset of subjects. Of the 91 participant events, 71 (56 border commuter events and 15 non-border commuter events) had personal 24-h measurements of 1-NP (Table 1).

Details of 1-NP and urine sample collection have been described elsewhere.[34] In brief, 1-NP was collected on a 37-mm PTFE Teflon filter with a 2 μm pore size (SKC, Eighty Four, PA, USA) using a fine particulate matter ($PM_{2.5}$) impactor (BGI HPEM, Waltham, MA, USA and SKC PEM, Eighty Four, PA, USA) connected to a personal air sampling pump (AirChek XR5000; SKC) operated at 4 l/min. For personal 1-NP samples, impactors were placed near the participants' breathing zone. Urine samples were collected in 500 ml polyethylene wide-

mouth bottles (VWR 16129-040; Radnor, PA, USA) for men and commode specimen containers (VWR 15704-116) for women. Both urine and 1-NP samples were immediately placed on ice and transferred to the San Diego State University School of Public Health laboratory and stored at −20 °C in a freezer. Before freezing, urine samples provided by women were transferred to the Nalgene polyethylene 500 ml bottles. Before analysis, all samples were shipped overnight on dry ice to the University of Washington and stored at −20 °C.

Sample Extraction, Quantification, and Analysis

1-NP in filter samples.—1-NP in air was measured as described by Miller-Schulze et al.[13] Sample extractions occurred in seven batches and each batch included two blank filters and two spiked filters to assess quality control. Extracts from all batches were quantified using two-dimensional high-performance liquid chromatography tandem mass spectrometry and were quantified in the same run to minimize between-day variability of the instrument. The average 1-NP concentration calculated to be present in the field blanks was 0.028 pg/m^3, with a standard deviation of 0.0071 pg/m^3. The effective limit of quantification (LOQ) was set to (blank+2 SD)/square root of 2, resulting in an LOQ of 0.030 pg/m^3. Concentrations below the LOQ were substituted with 0.030 pg/m^3. The accuracy and precision of the extraction and two-dimensional high-performance liquid chromatography tandem mass spectrometry was 80 ± 12% of the expected values as determined by looking at concentrations of 1-NP in spiked (fortified) filters.

Urinary 1-NP metabolites.—Urinary 1-NP metabolites were measured using an high-performance liquid chromatography tandem mass spectrometry method as described previously.[31] Urine volumes collected ranged from 20 to 482 ml, with an average of 165 ml. Extractions were optimized for 100 ml of urine. Of the 91 urine samples, 25 had volumes < 100 ml and thus were diluted with deionized water to bring the volume to 100 ml. The 1-NP metabolite concentrations (in pg metabolite per ml urine for a nominal 100 ml urine sample) calculated to be present in assay blanks were typically <0.02 pg/ml (calculated for a nominal 100 ml urine sample). These levels of contamination, while clearly distinguishable from the chromatographic baseline, were typically observed at the approximate level of the lowest calibration standard. The LOQ for the assay was set to (blank+2 SD)/sqrt2; 8-OHNP = 0.011 pg/ml and 8-OHNAAP = 0.014 pg/ml. These values were substituted for concentrations below the LOQ.

Fortified samples were prepared by spiking 100 ml deionized water with 25 ml of a standard solution containing 0.5 ng 8-OHNP and 1.0 ng 8-OHNAAP, along with the requisite deuterated internal standard spike analogous to that for the urine samples. The accuracy and precision of calculated concentrations of 1-NP metabolite species in the fortified samples were 8-OHNAAP 66 ± 6% and 8-OHNP 73 ± 6%.

Urinary creatinine levels were measured in the clinical laboratory at the University of Washington Medical Center using a colorimetric assay, and the creatinine measurements were used to adjust for diuresis. For each urine sample, 1 ml was held aside for this purpose,

except where the urine void volume was small and all the sample was used for the 1-NP metabolite analysis.

Statistical Analysis

The data set includes all samples for both 1-NP and its urinary metabolites, including the samples below the LOQ, which were substituted with (blank+2 SD)/sqrt2. Comparison of urinary concentrations between border commuters and non-border commuters was accomplished using a *t*-test. A multilevel linear regression model was performed on all study participants:

$$\text{Ln(Personal exposure to 1-NP)}_{ij} = \beta_0 + \beta_1 \text{Ln(metabolite)}_{ij} + \mu_i + e_{ij}$$

where $_i$ represents the participant, $_j$ represents a specific urine and filter sample pair, β_0 is the intercept parameter, β_1 is the slope estimate for the corresponding predictor variable (8-OHNP, 8-OHNAAP, or 8-OHNP+8-OHNAAP), and μ_i+e_{ij} is the random part of the model with the following distributions: $\mu_i \sim N(0, \gamma^2)$, $e_{ij} \sim N(0, \sigma^2)$

The results of the multilevel linear regression model were used to obtain an estimate of what percentage change in personal exposure to 1-NP was associated with the percent change in urinary metabolites.

In addition, within the subgroup of border commuters, we explored the association between urinary 1-NP metabolites and several predictor variables related to time spent commuting, time spent in proximity to roadways, and time spent at the border crossing. A list of the covariates used in the regression analysis can be found in Table 2. All the travel times are estimated from participants' time activity diary. All the covariates were continuous except for season, which was dichotomized into Spring/Summer (1 March to 31 August) and Autumn/Winter (1 September to 28 February). Season was tested as an interaction term for other predictor variables in the regression models. There were 47 border commuter events in the Autumn/Winter and 21 in the Spring/Summer.

We controlled for the effect of repeat participants in the regression analysis to minimize the possibility of any potential effect of more frequent repeated sampling of border commuters versus non-border commuters. Natural log transformations were used for all continuous variables. The Mann–Whitney *U*-test and all linear regression models used an α of $P < 0.05$ level of significance. Data analysis was performed using STATA/IC ver. 13.1(StataCorp LP, College Station, TX, USA).

RESULTS

Table 3 summarizes the descriptive statistics for the two 1-NP urinary metabolites and their sum for border commuter events and non-border commuter events, for both creatinine-adjusted and -unadjusted concentrations. The metabolite 8-OHNP was the most commonly detected in urine samples. For unadjusted 8-OHNP, 1/68 (1.5%) of border commuter events and 2/18 (11%) non-border commuter events were below the LOQ. For unadjusted 8-OHNAAP, 37/68 (54%) of border commuter events and 12/18 (66%) of non-border

commuter events were below the LOQ. Urinary 1-NP metabolite-unadjusted 8-OHNP concentrations were significantly higher for border commuters than non-border commuters, as were the sum of the metabolites (Table 3). Border commuters had approximately twofold higher levels of 8-OHNP compared with non-border commuters (mean concentration for unadjusted 0.076 *vs* 0.033 pg/ml; mean concentration for creatinine-adjusted 0.092 *vs* 0.042 pg/mg creatinine) (Table 3). Compared with non-border commuters, border commuters had threefold higher levels of unadjusted 8-OHNAAP (mean concentration 0.067 *vs* 0.021 pg/ml), and twofold higher levels of creatinine-adjusted 8-OHNAAP (0.065 *vs* 0.033 pg/mg creatinine) (Table 3). Summed mean metabolite concentrations were also 2-3 fold higher in border commuters compared to non-border commuters (Table 3).

Table 4 shows the linear regression results exploring the association between urinary 1-NP metabolites and personal exposure to 1-NP. The unadjusted models provide similar effect estimates for each metabolite and the sum of the metabolites estimating a 14% increase in 1-NP exposures for each 10% increase in 8-OHNP ($P = 0.3$), a 20% increase in 1-NP exposures for each 10% increase in 8-OHNAAP ($P = 0.02$), and a 16% increase in 1-NP exposures for each 10% increase in the sum of the metabolites ($P = 0.01$). Effect estimates were similar for the creatinine-adjusted models, and were modestly attenuated when data below the LOD were excluded.

Using multilevel linear regression modeling, for the subgroup of border commuters, we also examined the univariate predictors of urinary 1-NP metabolites listed in Table 2. Age and gender were not included in these models. In our sensitivity analysis age, as a continuous variable, had a small, nonsignificant effect on the effect estimate. Similarly, including gender in the models did not substantially change the effect estimate. Overall, these analyses did not indicate that the travel-related measures of exposure listed in Table 2 were informative predictors of urinary metabolite levels, with most effect estimates being small with wide confidence intervals encompassing zero. The time spent outdoors in Mexico and the United States was fairly equivalent (median: 216 min Mexico and 247 min United States). However, total time spent outdoors, including border commute, was small compared with the time spent at home (median: 67%). Border commuters spent a median of 60 min commuting the border per day. Season was the only significant predictor in the model for the unadjusted 8-OHNAAP metabolite ($\beta_1 = 0.049$; 95% confidence interval: 0.004–0.094), a finding that is consistent with our observation that border commuters' personal exposures to 1-NP were 60% higher in Winter *vs* Summer.[35]

DISCUSSION

This is the first study that compares urinary 1-NP metabolites with personal exposure to 1-NP to examine community exposure to DPM. In our study, we observed that border commuters had higher concentrations of two 1-NP metabolites, 8-OHNP, and 8-OHNAAP, as compared with non-border commuters, consistent with our previous report from the same sample that border commuters had higher personal 1-NP exposures compared with non-border commuters.[34] Median concentrations for personal 1-NP were 6.5-fold higher for border commuters (median, interquartile range: 0.96, 0.33–1.87 pg/m^3) compared with non-border commuters (median, interquartile range: 0.15 pg/m^3, 0.05–0.30 pg/m^3).[34] In addition,

higher urinary 1-NP metabolite concentrations were associated with higher personal 1-NP exposures: increases of 10% in urinary concentrations of 8-OHNP, 8-OHNAAP, or the sum of the two metabolites were associated with increases in personal 1-NP exposures over the prior 24 h of 14%, 20%, and 16%, respectively. However, the confidence intervals on these effect estimates were broad and the regression models explained only a small proportion of the variability in the relationship between 1-NP measured in the previous 24 h and the urinary metabolites.

Similarly, in his study of taxi drivers in Shenyang, China, Miller-Schulze et al.[31] also reported positive associations between urinary 1-NP metabolites and personal 1-NP exposures. However, the urinary concentrations of the 1-NP metabolites were not strongly predictive of the recent air exposure levels. There are several factors that could attenuate this relationship. For compounds that are eliminated relatively rapidly, a single spot urine sample may not be well correlated with longer-term exposures. Another potential source of variability that we were unable to address relates to inter-individual differences in metabolism that could attenuate the relationships between 1-NP and urinary biomarker levels.

The 8-OHNP metabolite levels we observed were at least 10-fold lower compared with those reported previously for highly exposed occupational cohorts,[14,31] and ~ 5-fold lower compared with the one previous report of a convenience sample of non-occupationally exposed participants (university students and faculty) from Kanazawa, Japan.[28] Nevertheless, we were able to distinguish differences in DPM exposure in the range of exposures taking place in the community.

A limitation of this study is that we were not able to quantify how much the border commute contributed to overall DPM exposure. The border commuters may have had additional exposure to DPM in Mexico, thus we cannot attribute the increase in 1-NP metabolites unequivocally to the exposure at the SYPOE. Additionally, participants who resided in Tijuana but who did not cross the border would have helped to resolve the contribution to 1-NP exposure associated with living in Tijuana from that associated with the border commute. Logistical considerations made this approach not feasible for the study reported here. In conclusion, we have demonstrated that urinary 1-NP metabolites were detectable in residents of the US–Mexico border communities of Tijuana, Mexico and San Diego, CA, and urinary concentrations were significantly higher in spot samples from the cohort of border commuters relative to a control group of non-border commuters, and were positively associated with measured personal exposures to 1-NP over the 24 h before urine collection. Overall, given the important adverse health impacts of community exposures to DE, a highly specific marker for DPM may prove useful to improve assessment of DE exposures. In spite of recent improvement in diesel engine technology that have markedly reduced DE emissions, including 1-NP, it is significant that 1-NP and its urinary metabolites were, nevertheless, readily detectable in this study of participants exposed to ambient concentrations of DE within their communities. With rising concerns about community level exposure to goods movement facilities, such as ports and freight rail yards, 1-NP and the corresponding urinary metabolites show promise as suitable markers of exposure to DE.

However, further studies are needed to better characterize sources of variability in the relationship between DE exposure and biomarker response.

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Table 1.

Summary of number of urine and personal 1-NP samples.[a]

No. of samples	Urine			Personal 1-NP			
	No. of non-border commuters	No. of border commuters	Total no. of participants	No. of samples	No. of non-border commuters	No. of border commuters	Total no. of participants
1	16	12	28	1	13	5	18
2	1	4	5	2	1	2	3
3		3	3	3		2	2
4		1	1	4		1	1
5		4	4	5		5	5
6		1	1	6		2	2
7		2	2				
Total participants, events	17, 18	27, 73	44, 91		14, 15	17, 56	71

Abbreviation: 1-NP, 1-nitropyrene.

[a]Successfully obtained from participants (total = 44 people and 91 measurement events).

Table 2.

Covariates used in regression analysis and their descriptions.

Covariate	Type	Description
Season	Categorical	Season was dichotomized into two groups: Spring/Summer (1 March to 31 August) and Autumn/Winter (1 September to 28 February). These cut-points were selected based on data showing that personal 1-NP concentrations were higher in the Autumn and Winter.[34] In addition to being used as a covariate, season was also tested as an interaction term as a result of seasonal differences in personal 1-NP concentrations.
Total travel	Continuous	Total amount of time in minutes that a participant spent on or near a roadway in the US and Mexico.
Total travel border	Continuous	Total amount of time in minutes that a border crosser spent in line waiting to cross northbound across the US-Mexico San Ysidro Port of Entry.
Total travel US	Continuous	Total amount of time in minutes that a participant spent on or near a roadway in the US.
Total travel US | Season	Continuous	Total amount of time in minutes that a participant spent on or near a roadway in the US during Spring/Summer or Autumn/Winter.
Total travel Mexico	Continuous	Total amount of time in minutes that a participant spent on or near a roadway in Mexico.
Total travel Mexico | Season	Continuous	Total amount of time in minutes that a participant spent on or near a roadway in Mexico during Spring/Summer or Autumn/Winter.
$C_{border} \times T_{border}$	Continuous	Concentration of 1-NP (pg/m^3) (mean (SD) = 2.0 (2.0)) collected at the border during the participants north bound border crossing multiplied by the amount of time in minutes the participant spent in line going northbound at the US–Mexico border at San Ysidro.
$C_{border} \times T_{border}$ | Season	Continuous	Concentration of 1-NP (pg/m^3) collected at the border during the participants north bound border crossing multiplied by the amount of time in minutes the participant spent in line going northbound at the US-Mexico border at San Ysidro during Spring/Summer or Autumn/Winter.
$C_{home} \times T_{home}$	Continuous	Concentration of 1-NP (pg/m^3) (border commuters: mean (SD) = 0.64 (0.81); non-border commuters: mean (SD) = 0.072(0.079)) collected inside the participants home multiplied by the amount of time in minutes the participant spent inside their home.

Abbreviation: 1-NP, 1-nitropyrene.

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Table 3.

Comparison of urinary 8-OHNP and 8-OHNAAP levels.[a]

	n[b]	*Mean (SD)*	*GM (GSD)*	*Range*	P-*value*[c]	n *(% < LOQ)*[d]
8-OHNP						
Unadjusted 8-OHNP (pg/ml) in urine						
Border commuter events	68	0.076 (0.066)	0.058 (0.059)	0.011–0.37	<**0.01**	1 (1.5)
Non-border commuter events	18	0.033 (0.020)	0.027 (0.020)	0.011–0.088		2 (11)
Creatinine-adjusted 8-OHNP (pg/mg creatinine) in urine						
Border commuter events	50	0.092 (0.12)	0.057 (0.098)	0.010–0.71	0.07	1 (2)
Non-border commuter events	17	0.042 (0.030)	0.035 (0.029)	0.016–0.13		2 (12)
8-OHNAAP						
Unadjusted 8-OHNAAP (pg/ml) in urine						
Border commuter events	68	0.067 (0.110)	0.032 (0.095)	0.014–0.56	0.07	37 (54)
Non-border commuter events	18	0.021 (0.014)	0.019 (0.013)	0.014–0.060		12 (66)
Creatinine-adjusted 8-OHNAAP (pg/mg creatinine) in urine						
Border commuter events	50	0.065 (0.092)	0.035 (0.078)	0.0047–0.57	0.15	26 (52)
Non-border commuter events	17	0.033 (0.025)	0.026 (0.024)	0.087–0.10		11 (67)
8-OHNP+8-OHNAAP						
Unadjusted 8-OHNP+8-OHNAAP (pg/ml) in urine						
Border commuter events	68	0.14 (0.13)	0.11 (0.11)	0.025–0.66	<**0.01**	1 (1.5)
Non-border commuter events	18	0.054 (0.026)	0.048 (0.025)	0.025–0.10		2 (11)
Creatinine-adjusted 8-OHNP+8-OHNAAP (pg/mg creatinine) in urine						
Border commuter events	50	0.16 (0.15)	0.11 (0.12)	0.024–0.76	**0.024**	1 (2)
Non-border commuter events	17	0.075 (0.051)	0.063 (0.047)	0.031–0.23		2 (12)

Abbreviations: GM, geometric mean; GSD, geometric standard deviation; LOQ, limit of quantification; 8-OHNAAP, 8-hydroxy-*N*-acetyl-1-aminopyrene; 8-OHNP, 8-hydroxy-1-nitropyrene.

[a]Between border commuter and non-border commuters, before and after adjusting for creatinine.

[b]Number of participant events.

[c]Bold indicates value was $P < 0.05$.

[d]*n* < LOQ, number of urine samples below LOQ of 0.011 pg/ml for 8-OHNP and 0.014 pg/ml for 8-OHNAAP. Samples below and above LOQ were included in the analysis.

Table 4.

Univariate analysis using multilevel linear regression modeling.[a]

Model parameter	n[b]	β_1	*CI*	P-*value*[c]	n (%<LOQ)[d]
8-OHNP					
Unadjusted	68, 31	1.4	-1.2, 4.0	0.30	1 (1.5)
Creatinine adjusted	49, 27	1.3	-1.3, 3.9	0.31	1 (2)
8-OHNAAP					
Unadjusted	68, 31	1.9	0.35, 3.5	**0.02**	26 (45)
Creatinine adjusted	49, 27	1.9	-0.26, 4.0	0.08	7 (22)
8-OHNP+8-OHNAAPP					
Unadjusted	68, 31	1.8	0.41, 3.1	**0.01**	3 (4.5)
Creatinine adjusted	49, 27	1.6	-0.002, 3.3	0.05	3 (6.5)

Abbreviations: CI, confidence interval; LOQ, limit of quantification; 8-OHNAAP, 8-hydroxy-*N*-acetyl-1-aminopyrene; 8-OHNP, 8-hydroxy-1-nitropyrene; 1-NP, 1-nitropyrene.

[a]To examine association between urinary 1-NP metabolites and personal exposure to 1-NP (pg/m^3) for all study participants. Accounted for repeat samples in the modeling.

[b]Number of border commuter events, number of participants.

[c]$P < 0.05$ in bold.

[d]*n* < LOQ, number of urine samples below limit of quantification. Samples below and above LOQ were included in analysis.