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ORIGINAL ARTICLE

Evaluation of urinary metabolites of 1-nitropyrene as biomarkers for exposure to diesel exhaust in taxi drivers of Shenyang, China

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Diesel exhaust (DE) is a significant contributor to the toxicity associated with particulate matter (PM). 1-Nitropyrene (1-NP) has been used as a molecular marker for DE, and the urinary metabolites of 1-NP have been proposed as biomarkers for exposure to DE. In this study, several urinary 1-NP metabolites were evaluated for their utility as markers of short-term exposures to DE. The study population was a cohort of 24 taxi drivers from Shenyang, China, who submitted urine samples collected before, after, and the next morning following their workshifts. The urinary metabolites studied were isomers of hydroxy-1-nitropyrene (3-, 6-, 8- OHNPs) and hydroxy-*N*-acetyl-1-aminopyrene (3-,6-, 8-OHNAAPs). Exposure to DE was estimated based on exposure to 1-NP in air samples collected during and after the driver's workshift; 6- and 8-OHNP, and 8-OHNAAP were consistently detected in the drivers' urine. Concentrations of the metabolites in the taxi drivers' urine were greater than metabolite levels previously reported in non-occupationally exposed subjects; however no associations were observed between subject-specific exposures to 1-NP and urinary metabolites measured at the end of the workshift or in the next morning void. Significant autocorrelation was observed in metabolite levels in successive urine samples, from which half-lives for urinary elimination of $\sim$10–12 h were estimated. These observations suggest that, in an occupational setting, urinary 1-NP metabolites may be more suitable as markers of ongoing exposure (timescales of several days) rather than indicators of acute exposure associated with single workshifts.

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INTRODUCTION

Particulate matter (PM) is a well-chronicled hazard to human health. Fine PM, or PM of aerodynamic diameter of $<2.5\ \mu$m ($PM_{2.5}$), is hazardous because of both its size and chemical composition, which are attributes of the PM source. Diesel exhaust (DE), as diesel particulate matter (DPM), is a significant contributor to ambient PM, particularly $PM_{2.5}$.[1] In addition, DPM is of significant concern in terms of its health effects, as it has been shown to cause cardiac and respiratory disease,[1] as well as being classified as a possible human carcinogen (IARC type 2B).[2] Nitropolycyclic aromatic hydrocarbons (NPAHs) are formed by incomplete combustion of fossil fuels, and certain NPAH species, including 1-nitropyrene (1-NP), are present at elevated levels in the PM emitted from diesel engines relative to either other combustion sources or ambient PM.[3] 1-NP has been suggested as a molecular marker for exposure to DE and DPM owing to this enrichment, as well as its potential applicability as a biomarker for DE exposure through the quantification of its metabolites in human urine.[3–5]

The assessment of sources of $PM_{2.5}$ and personal exposures to $PM_{2.5}$ is a well-established and continually growing field of environmental research that involves a wide variety of methodologies, including receptor-based modeling, personal filter sampling, and use of regional monitoring stations to estimate exposures.[6] Accurate assessment of individual exposures to any pollutant of interest, including DE, is challenging owing to both the existence of variable concentrations in the ambient environment as well as differences in breathing rate and particle retention among the exposed human population.[1] Consequently, biomarkers of exposure, that is, compounds measured in biological specimens subsequent to exposure to a pollutant/source of interest (either as metabolites of the pollutant or as a biological response to the exposure) have garnered significant interest owing to their potential as quantitative measures of an individual's exposure to and uptake of pollutants and/or sources of interest.[7]

Urinary metabolites of 1-NP have been previously proposed as biomarkers of DE exposure.[5] 1-NP is metabolized in the body primarily via two pathways: cytochrome P450-mediated C oxidation and nitroreduction,[5] and the metabolites typically observed (either in the feces or the urine) are the hydroxy-1-nitropyrenes (3-, 6-, and 8-OHNPs), the hydroxy-*N*-acetyl-1-aminopyrenes (3-, 6-, and 8-OHNAAPs), *trans*-4,5, dihydro-4,

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5-dihydroxy-1-nitropyrene, *N*-acetyl-1-aminopyrene (NAAP), and 1-aminopyrene (1-AP).[8–10] In a study in which rats were dosed intragastrically with a DPM standard reference material, van Bekkum et al.[11] found that 7% of the administered dose of 1-NP was excreted in the urine as 6-OHNAAP, while 1.6% was excreted as 6-OHNP within 12 h of dosing. In the recent study of 1-NP metabolites in urine from subjects with environmental exposure to DE by Toriba et al.,[5] 2 of 3 of the OHNAAP isomers (6- and 8-OHNAAP) and 2 of 3 of the OHNP isomers (6- and 8-OHNP) were detected in 100% of the samples.

A study of the particle-associated NPAH exposure of taxi drivers in Shenyang, China, was published previously.[12] In that study, taxi drivers were monitored for a 24-h period encompassing their workshift (in which they were exposed to elevated levels of traffic-related pollutants, including DE) and their non-workshift home exposure. A component of the sample collection for that study was to collect urine samples from the drivers along with the PM samples. In this paper, the analysis of these urine samples for 1-NP metabolites and the association between metabolite levels and 1-NP exposure is reported.

MATERIALS AND METHODS

Sampling

Twenty-four taxi drivers were recruited for this study, which took place in August 2007. The taxi driver subjects consisted of 23 males and 1 female, with a mean age of 42 years (range: 29–51 years). Each driver was monitored for a single 24-h period, and six drivers were monitored per day during the 4-day sampling period. The vehicles driven by the subjects were a mix of diesel- and gasoline/natural gas hybrid-powered cars. Three urine samples were collected for each subject: a ''pre-shift'' first morning void, a ''post-shift'' sample, and a ''next-day'' sample (first morning void from morning subsequent to the day the drivers were monitored). Of the 72 urine samples collected, 12 had volumes <100 ml. Drivers were provided with identical lunches to help control for dietary exposures to 1-NP. Samples were collected in polyethylene (HDPE) bottles and stored at $-20\,^{\circ}C$ before analysis.

Personal air monitoring for PM, EC, and selected NPAHs was undertaken over a 24-h period contemporaneous with the urine samples, and has been reported in detail in an earlier publication.[12] In brief, $PM_{10-2.5}$ and $PM_{2.5}$ were collected inside and outside the drivers' taxis during their workshift, and at their homes from the end of their workshift until the next morning. These filter samples are referred to as ''Inside Car'', ''Outside Car'', and ''Home'', respectively.

Materials

Chemical reagents for sample preparation and analysis were HPLC grade and purchased from Sigma-Aldrich, St. Louis, MO, USA or JT Baker, Mallinckrodt Baker, Phillipsburg, NJ, USA. β-Glucuronidase/arylsulfatase (*Helix pomatia*) was obtained from Roche Diagnostics, Indianapolis, IN, USA (100,000 Fishman units/mL/800,000 Roy units/mL, Roche, 127 698). Blue Rayon was obtained from MP Biomedicals, Solon, OH, USA and Funakoshi Company, Tokyo, Japan. Alumina A solid-phase clean-up cartridges were obtained from Waters, Millford, MA, USA. All 1-NP metabolite standards were synthesized in our laboratory as reported previously.[5]

Urine sample preparation

The sample preparation used to extract the 1-NP metabolite compounds from the Shenyang taxi drivers' urine samples was similar to that used in the study by Toriba et al.,[5]. but will be described here briefly as some components differ. Urinary creatinine levels were measured using a colorimetric assay and the creatinine measurements were used to adjust for diuresis-1 ml of each urine sample was held aside for this purpose. In all, 100 ml of urine was vacuum filtered through nylon-membrane filters (pore size 0.45 μm) and transferred to silanized glass bottles (if <100 ml was available, that volume was held aside and analyzed in its entirety). The suite of deuterated internal standard compounds were then added to each urine sample (200–2000 pg per compound). In addition, 5 ml of 4M sodium acetate buffer and 75 μl 1M HCl were added to adjust the pH to 5. In order to hydrolyze the native-conjugated 1-NP metabolites, 75 μl β-glucuronidase/aryl sulfatase was also added to each sample. The samples were then incubated at 37 °C for 4 h. After the incubation, 100 mg Blue Rayon was added to each sample and the samples were shaken at room temperature for 1 h in the dark to extract the deconjugated metabolites from the urine. The urine was poured through a polypropylene SPE cartridge with a polypropylene frit (20 μM porosity), attached to a SPE vacuum manifold. The Blue Rayon was rinsed twice with a small volume of water, and the rinse and urine were discarded. The 1-NP metabolites were extracted from the Blue Rayon with 20 ml 50:1 methanol:ammonia (v:v) and 30 min of sonication. The methanol/ammonia extract was then decanted into Turbovap tubes and 50 μl of DMSO was added as a ''keeper solvent''. This liquid mixture was then evaporated to near dryness under a stream of nitrogen in a Turbovap evaporative concentrator at 45 °C. The residue was reconstituted in 5 ml 1:1 ethyl acetate:methanol (v:v) and then run through a Sep-Pak Alumina A cartridge. The Turbovap tubes were rinsed with an additional 10 ml of the ethyl acetate : methanol (v:v) solvent mixture followed by a further 5 ml of methanol. This 20 ml of solvent was then evaporated in the Turbovap evaporative concentrators at 45 °C, again to near dryness, and reconstituted in 300 μl methanol. This 300 μl methanol was filtered through a polypropylene syringe with a 0.45-μm pore size PTFE syringe filter into a silanized glass autosampler vial insert. This volume was reduced to 50 μl under nitrogen, and 20 μl water were added, bringing the final extract volume to 70 μl. In all, 10 μl of this sample extract solution was then analyzed via the LC-MS/MS method as described below.

Analytical method for determination of 1-NP metabolites

The sample extracts, obtained as described above, were analyzed using an HPLC-MS/MS method based on that developed by Toriba et al.[5]. The method utilized an Agilent 1100 HPLC system (binary pump, mobile phase degasser, autosampler, heated column compartment, and a triple quadrupole mass spectrometer). The injected volume was 10 μl. The mobile phases were (A) water and (B) methanol; each with 0.01% ammonia. The mobile phase gradient was as follows: initially 30% B, gradient to 50% B over 15 min, step to 80% B and hold until 21 min, step to 30% B and hold until 27 min. The nm column used was an Agilent Zorbax Extend-C18, 2.1 × 100 mm with 3.5-μm particle size. Column temperature was 30 °C. The mobile phases were kept in ice during the analysis to slow evaporative loss of ammonia.

The mass spectrometer (MS) used in this method was an Agilent Model 6410 triple quadrupole mass spectrometer (Agilent Technologies Santa Clara, CA, USA) equipped with an electrospray ionization (ESI) source. The ESI was used in negative mode and the source and MS parameters were set as described in the Supplementary Information (SI). The resolution of the first and third mass analyzers was set on ''wide resolution'' and the system was operated in multiple reaction mode (MRM). The transitions monitored to quantify the levels of 1-NP metabolites were as follows: for the OHNAAPs, the transition for the native analytes was *m/z* 274→231 (corresponding to loss of $COCH_3$, 43 amu); for the deuterated internal standards for the OHNAAPs (d_8), the transition monitored was *m/z* 282→239. For the OHNPs, the transition monitored was *m/z* 262→232 (loss of NO, 30 amu); for the deuterated internal standards for the OHNPs (d_8), the transition monitored was *m/z* 270→240. For NAAP, the transition monitored was *m/z* 258→216 (loss of $COCH_2$, 42 amu); for the deuterated internal standards for the NAAP (d_9), the transition monitored was *m/z* 267→225.

Quantification of determinants

Calibration solutions with 1-NP metabolite concentrations ranging 0.71–58 pg/μl were injected with each analysis sequence. Deuterated 1-NP metabolite internal standard compounds were added to each calibrant solution at a fixed concentration equivalent to their expected concentrations in the sample extracts. The concentrations of these deuterated internal standards are listed in Supplementary Table SI-2 of

the SI. Selected calibration standards were analyzed periodically throughout the sequence to monitor instrument performance. The ratio of the analyte peak area to the peak area of the corresponding internal standard versus the concentration of the analyte in the calibrant solution was plotted and a linear regression equation was created with 1/X weighting for each analyte. Because the deuterium-labeled internal standards are carried through the entire extraction and analysis process, suffering the same degree of losses as the native compounds, the use of the relative ratio of analyte response to internal standard response to calculate analyte concentration enables accurate and precise concentrations to be determined for the native analytes despite low and variable recoveries of both the analytes and internal standards from the urine samples (see subsequent paragraphs of this section). In addition, the calculated concentration of each metabolite in the urine samples was divided by the creatinine concentration to correct for differences in hydration between subjects.

Quality control (QC) samples were included with each batch of urine samples to give an insight on assay performance and blank contamination. The relevant results of these QC analyses are summarized as follows. The average 1-NP metabolite concentrations (in pg metabolite/ml urine for a nominal 100 ml urine sample) calculated to be present in assay blanks were as follows (mean ± standard deviation): 6-OHNP = 0.06 ± 0.01 and 8-OHNP = 0.04 ± 0.02. These levels of contamination, while clearly distinguishable from the chromatographic baseline, were typically observed at the approximate level of the lowest calibration standard. No blank contamination was detected for 3-OHNP, 3-OHNAAP, 8-OHNAAP, or 6-OHNAAP. For the purposes of this study, "assay blanks" are defined as aliquots of 100 ml of deionized water spiked with the same amount of deuterated internal standards as the samples, and processed in the same manner as the samples. Comparison of the assay blanks with subsequent data for the 1-NP metabolite concentrations in taxi drivers' urine samples (6-OHNP 2.51 ± 4.02 pg/ml; 8-OHNP 2.10 ± 3.23 pg/ml) shows that the blank contamination is negligible for 6-OHNP and 8-OHNP. In addition, in the case of 6- and 8-OHNP, the lowest detected concentration in all the urine samples was greater than average level in the assay blanks plus 1 standard deviation. However, a substantial contaminant peak with a retention time similar to 1-NAAP was present in the assay blanks; therefore, no data is presented for 1-NAAP.

Fortified samples were prepared by spiking 100 ml deionized water with 25 μl of a solution of OHNAAPs, OHNPs, and NAAP at concentrations of 20.0 pg/μl (3-, 6-, 8-OHNP, and NAAP), 6.20 pg/μl (3-OHNAAP), 38.2 pg/μl (8-OHNAAP), and 52.0 pg/μl (6-OHNAAP), along with the requisite deuterated internal standard spike analogous to that for the urine samples. The average recovery of 1-NP metabolites, as measured by dividing the measured instrumental response for the deuterated internal standards in these fortified samples with the average response for the deuterated internal standards in the two "control" samples (vial inserts spiked with an equivalent amount of deuterated internal standards and diluted appropriately with extract matrix) analyzed in that batch was: 8-OHNAAP = 21 ± 7%, 6-OHNP = 33 ± 8%, and 8-OHNP = 33 ± 9%. Recoveries for 6-OHNAAP and 3-OHNAAP were low and variable; therefore, no data is reported for these two compounds. The accuracy of calculated concentrations of 1-NP metabolite species in the fortified samples were 8-OHNAAP 87 ± 14%, 6-OHNP 109 ± 35%, 8-OHNP 92 ± 14%, and 3-OHNP 91 ± 11%. The use of isotope dilution with an internal standard for quantification, an approach used for this type of analysis by Toriba et al.[5] among others yields acceptable values of accuracy for the 1-NP metabolites in the fortified samples (described above) despite the somewhat low and variable recoveries of the deuterated internal standards.

RESULTS AND DISCUSSION

Specific 1-NP metabolites were detected in all urine samples. These data are summarized in Table 1, and sample chromatograms obtained from a calibration standard and a taxi driver urine sample are shown in Figure 1a and 1b, respectively. Although the LC-MS/MS method described previously was developed to have the capability to provide quantitative data for seven urinary 1-NP metabolites (3-, 6-, and 8-OHNAAP, 3-, 6-, and 8-OHNP, and NAAP) in human urine samples, not all of these metabolites were reliably or consistently detected in urine samples collected from the Shenyang taxi drivers.

As shown in Figure 1b, 8-OHNAAP, 6-OHNP, and 8-OHNP are clearly observed and can be reliably quantified. Several large unidentified peaks are present in the chromatogram, one of which partially obscures the peak attributed to 3-OHNP and prevents reliable quantification of 3-OHNP. 6-OHNAAP is not present at a high enough level to be reliably detected and quantified above the background noise in the chromatogram. Because 1-NAAP was detected in assay blanks at concentrations similar to those observed in the urine samples (~0.1 pg/ml urine), we were unable to reliably measure 1-NAAP in the urine samples.

The recoveries of the deuterated internal standard compounds from the urine samples were fairly similar to the recoveries reported for fortified samples in the methods section. The average recoveries for the three 1-NP metabolites reported in Table 1 were

Table 1. Creatinine-corrected 1-NP metabolite concentrations (pg/mg creatinine) in Shenyang taxi drivers.

		6-OHNP	*8-OHNP*	*8-OHNAAP*
Pre-shift	*N*	22	22	19
	Mean (SD)	1.9 (1.8)	1.8 (1.7)	0.31 (0.28)
	Median	1.4	1.3	0.17
Post-shift	*N*	23[a]	22[a]	23
	Mean (SD)	1.3 (1.1)	1.5 (1.1)	0.26 (0.15)
	Median	0.96	1.1	0.23
Next AM	*N*	23	23	24
	Mean (SD)	1.8 (1.6)	1.9 (1.5)	0.27 (0.16)
	Median	1.2	1.2	0.23
Cross-shift change[b]	*N*	21	20	18
	Mean (SD)	−0.48 (1.7)	−0.18 (1.6)	−0.05 (0.24)
	Median	0.03	0.18	−0.01
Overall	*N*	68	67	65
	Mean (SD)	1.68 (1.52)	1.74 (1.45)	0.28 (0.2)
	Median	1.22	1.24	0.23

[a]For the 6- and 8-OHNP 1-NP metabolites in the "post" and "cross-shift change" samples, a high-outlier (by Dixon's Q-test) was excluded from the summary statistics.
[b]Cross-shift change calculated as post-shift concentration minus pre-shift concentration.

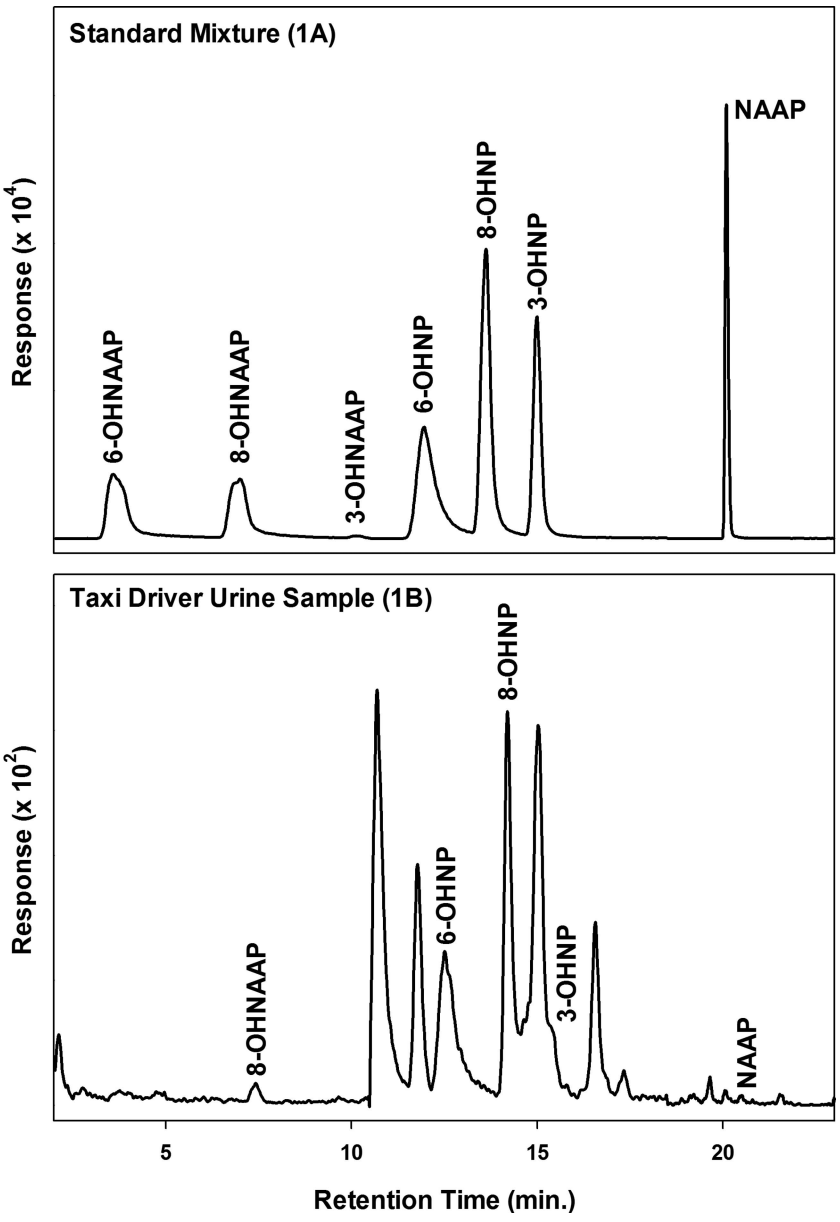

Figure 1. a and b: HPLC-MS/MS chromatogram (in multiple-reaction-monitoring, or MRM, mode) of 1-NP metabolite standard mixture (1a, top pane) and Shenyang taxi driver urine sample (1b, bottom pane). All three MRM transitions (for the OHNAAPs, OHNPs, and NAAP) are plotted in the same pane for both samples). Both chromatograms on same scale with respect to the X axis. Compounds labeled in figure, see text for abbreviations. Note that 3-OHNP appears as a shoulder on the side of a larger, interfering peak and cannot be reliably quantified.

as follows (mean ± standard deviation): 8-OHNAAP 16 ± 5%, 6-OHNP 29 ± 13%; 8-OHNP 32 ± 15%.

As shown in Table 1, the concentrations of 6- and 8-OHNP are significantly greater than the concentrations of the other 1-NP metabolite reported, 8-OHNAAP. This is consistent with the work of Toriba et al.,[5] who also measured higher levels of 6- and 8-OHNP in the urine of Japanese study subjects, although the difference observed in that study was not as pronounced as that in the current study. Studies in our laboratory from other human cohorts also show a predominance of OHNPs over OHNAAPs in urine samples.[13] By contrast, van Bekkum et al.[14] reported that rats exposed to DE particles via inhalation preferentially excreted OHNAAP metabolites.

The data in Table 1 also indicate that the urinary 1-NP metabolite concentrations are similar in pre-shift, post-shift, and next day urine samples. Paired *t*-tests confirm that there were no statistically significant differences between the three urine samples for each of the 1-NP metabolites listed in Table 1.

We also explored correlations between urinary concentrations of the different 1-NP metabolites. As shown in Table 2, 6-OHNP and 8-OHNP are highly correlated with each other. Concentrations of 8-OHNAAP were not consistently correlated with the other two metabolites. It is possible that a significant factor in the strong correlation of 6-OHNP and 8-OHNP is related to both isomers being formed during the same step of the metabolic transformation of 1-NP[5]. By contrast, formation of 8-OHNAAP requires additional metabolic transformation. Inter-individual differences in either *N*-acetyltransferase activity, or the nitroreduction of 1-NP and its metabolites, could be responsible for 8-OHNAAP concentrations varying independently of the OHNP metabolites.

The data in Table 2 also indicate a high degree of auto-correlation among successive urine samples. That is, concentrations

Table 2. Correlations (Pearson's *r*, one-tailed) among urinary 1-NP metabolites.

	Post-6-OHNP	*Next AM 6-OHNP*	*6-OHNP cross-shift change*	*Pre-8-OHNP*	*Post-8-OHNP*	*Next AM 8-OHNP*	*8-OHNP cross-shift change*	*Pre-8-OHNAAP*	*Post-8-OHNAAP*	*Next AM 8-OHNAAP*	*8-OHNAAP cross-shift change*
Pre-6-OHNP	**0.388**	**0.372**	**−0.799**	**0.959**	**0.382**	0.358	**−0.779**	0.279	0.307	0.051	−0.103
Post-6-OHNP		**0.602**	0.244	**0.429**	**0.966**	**0.513**	0.217	0.033	0.072	0.013	−0.082
Next AM 6-OHNP			0.015	**0.403**	**0.539**	**0.975**	−0.017	0.278	0.33	**0.492**	−0.102
6-OHNP cross-shift change				**−0.729**	0.207	−0.003	**0.953**	−0.255	−0.304	−0.047	0.037
Pre-8-OHNP					**0.454**	**0.399**	**−0.772**	0.32	0.332	0.151	−0.147
Post-8-OHNP						**0.478**	0.216	0.108	0.217	0.156	−0.145
Next AM 8-OHNP							−0.033	0.327	0.322	**0.564**	−0.151
8-OHNP cross-shift change								−0.242	−0.274	−0.115	0.043
Pre-8-OHNAAP									**0.51**	0.303	**−0.799**
Post-8-OHNAAP										**0.361**	0.109
Next AM 8-OHNAAP											−0.126

Significant correlations ($P > 0.05$) highlighted in bold.

of a given metabolite in the pre-shift urine sample are highly correlated with the concentrations of the same metabolite in the post-shift and next morning samples. This reflects a high degree of between-subject variability in biomarker levels compared with within-subject variability. Two-way ANOVA also found that for each metabolite, metabolite concentrations were significantly different between subjects ($P < 0.05$), but not between samples for the same subject. Possible explanations for this observation include significant inter-subject differences in 1-NP exposure or 1-NP metabolism. An alternative possibility is that the half-life for urinary elimination of 1-NP is relatively long compared with the time between collection of urine samples. This possibility will be discussed later in the manuscript.

Table 3. Correlations (Pearson's *r*) between urinary 1-NP metabolites and work-shift exposure to 1-NP.

	6-OHNP	*8-OHNP*	*8-OHNAAP*
Post-shift	0.137	0.117	0.084
Next-day	−0.035	−0.061	−0.275
Cross-shift change	0.213	0.168	**0.420**[a]

[a]Correlation is significant at the 0.05 level.
P-values calculated using 1-tailed test for significance.

Correlation of 1-NP exposures and 1-NP metabolite concentrations in urine

A major objective of the current study was to explore the feasibility of using the metabolites of 1-NP as biomarkers for exposure to DE. Therefore, the correlation between the taxi drivers' 1-NP exposures and the concentrations of 1NP metabolites in the urine samples collected from these subjects was of particular interest. For each of the three metabolites in Table 1, we calculated the association between workshift exposure to 1-NP and (i) metabolite concentrations in post shift urine, (ii) metabolite concentrations in the next morning urine sample, and (iii) cross-shift change in the urinary metabolite concentrations. As described previously, airborne concentrations of 1-NP during the drivers' workshift were approximately fourfold higher than non-workshift concentrations.[12] Coupled with the relatively long workshift duration (~8–10 h), workshift exposure to 1-NP would represent the bulk of the subjects' total 1-NP exposure.

Correlations between urinary metabolite levels and 1-NP exposure are shown in Table 3. A clear relationship was not observed between the 1-NP exposures and the urine samples collected from the taxi drivers subsequent to these exposures. One possible reason for our failure to detect a consistent relationship between personal exposure to 1-NP and urinary metabolite concentrations may be that the relatively short-term time course of the study (i.e., sample collection immediately after exposure to 1-NP) was too short to capture the 1-NP metabolites produced in response to the measured exposure. As few studies are available from which to estimate a half-life for urinary 1-NP metabolites in humans, we attempted to estimate this elimination half-life based on the correlations between 1-NP metabolite levels in successive urine samples in the current study. Assuming (i) that elimination of 1-NP is a first-order process, and (ii) ongoing random exposure to 1-NP, the autocorrelation coefficient (r) is directly related to the time constant for urinary elimination (τ), and the half-life for urinary elimination ($t_{1/2}$) is as follows:

$$r(\Delta t) = \exp(-\Delta t)/\tau \quad (1)$$

$$(t_{1/2}) = \ln(2)\tau \quad (2)$$

where Δt is the time interval between successive urine samples. We used a boot-strap approach to estimate τ from the 1-NP metabolite concentrations in the taxi drivers' urine samples. The base sample population consisted of ~41–45 pairs of pre-shift–post-shift and post-shift–next-am urine measurements. For each analyte, we then created 1000 new data sets by sampling with replacement 41–45 pairs of successive urine concentrations. For each of the 1000 simulated data sets, the autocorrelation coefficient was calculated, and used via eqns.1&2 to calculate $t_{1/2}$. We used a fixed value of 12 h for Δt, which is intermediate between the average Δt between pre-shift and post-shift measurements (11.4 ± 0.6 h) and the average Δt between post-shift and next-am measurements (12.2 ± 0.4 h) in the taxi driver cohort. Mean values of $t_{1/2}$ (± 95% confidence interval) calculated as described above were as follows: 6-OHNP 10.6 ± 0.7 h, 8-OHNP 11.1 ± 0.7 h, and 8-OHNAAP 11.7 ± 1.0 h. Because the subjects in our study were small in number, predominantly male, represented a limited age range (29–51 years) and of a single ethnicity, the confidence intervals we report for the urinary elimination half-life may underestimate the full range of elimination half-lives observed in a more heterogeneous population.

As mentioned above, there are no existing studies that report half-lives for urinary elimination of 6-OHNP, 8-OHNP, and 8-OHNAAP in humans. However, Laumbach et al.[4] did explore urinary elimination of 1-aminopyrene (1-AP) following exposure of human subjects for 1 h to a DE atmosphere containing 2680 pg/m^3 1-NP. Although the authors did observe that urinary levels of 1-AP were consistently elevated following the DE exposure, relative to a filtered air exposure, the rates of urinary elimination of 1-AP was highly variable. A second study by these researchers found that

Table 4. Comparison of urinary 1-NP metabolite concentrations (mean ± SD, pg/mg creatinine) with other studies.

	6-OHNP	*8-OHNP*	*8-OHNAAP*
Shenyang, China (taxi drivers, current study)	1.68 ± 1.52	1.74 ± 1.53	0.28 ± 0.20
Kanazawa, Japan (college students[5])	0.42 ± 0.24	0.28 ± 0.14	0.26 ± 0.22
Seattle, USA (Miller-Schulze, unpublished data)	0.56 ± 0.70	0.57 ± 0.69	0.11 ± 0.14

while the majority of subjects excreted the maximum concentration of 1-NP metabolite in a relative short time period (~5 h), a significant fraction (almost 1/3) of the subjects were estimated to excrete the maximum metabolite concentration at a time that exceeded 24-h post-exposure.[15] Although the metabolite studied by Laumbach et al, 1-AP, is not the same compound as those measured in the current work, (in fact, 1-AP is formed in the body via a different combination of metabolic pathways, as compared with the OHNP and OHNAAP metabolites), our estimates of urinary elimination rates for OHNP and OHNAAP metabolites are broadly consistent with the range of values observed by them.

To date, there are very few published measurements of 1-NP metabolite concentrations in human urine. The studies that reported urinary levels of 8-OHNAAP, 6-OHNP, and 8-OHNP are summarized in Table 4. Two other studies reported levels on 1-AP in human urine, but did not measure the hydroxylated metabolites.[4,16]

Considering the data in Table 4, levels of 6-OHNP and 8-OHNP are dramatically higher in the occupationally exposed taxi drivers from the current study, compared with non-occupationally exposed subjects in Kanazawa, Japan and Seattle, USA. Although personal exposure data are not available for the Seattle and Kanazawa subjects, it is expected that these groups would have substantially lower 1-NP exposures compared with the taxi drivers. The higher urinary 6-OHNP and 8-OHNP concentrations in the taxi drivers relative to the other two groups are consistent with the anticipated difference in exposures. By contrast, urinary concentrations of 8-OHNAAP were not notably different among the three groups, and do not appear to be associated with the expected differences between the groups in 1-NP exposures.

In conclusion, our study of 1-NP metabolites in urine samples from taxi drivers in Shenyang, China, in August 2007, showed that selected 1-NP metabolites (8-OHNAAP, 6-OHNP and 8-OHNP) were detected and quantified in essentially all of the 72 samples collected over the 4-day period. Interfering compounds present in the sample extracts prevented reliable quantification of the two of the other targeted metabolites (NAAP and 3-OHNP). Our data showed that the concentrations of 6- and 8-OHNP were highly correlated over all shift types and drivers. Statistically significant autocorrelation was also observed for each of the NP metabolites, suggesting that elimination half-lives were relatively long in comparison to the elapsed time between collection of successive urine samples. Elimination half-lives of 10–12 h were estimated based on the autocorrelation coefficient. Perhaps as a consequence of these relatively long elimination half-lives, no correlation was observed between the drivers' workshift exposures to 1-NP and urinary metabolite concentrations measured either at the end of the work shift, or in the next morning void. Absolute concentrations of 1-NP metabolites in urine samples from these occupationally exposed subjects were, however, higher than previous reports of the same metabolites in non-occupationally exposed subjects. These observations suggest that in an occupational setting, urinary 1-NP metabolites may be more suitable as markers of ongoing exposure (timescales of several days) rather than indicators of acute exposure associated with single workshifts.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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