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Method development for the measurement of quinone levels in urine

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ABSTRACT

A method was developed for the quantification of 1–4 ring quinones in urine samples using liquid–liquid extraction followed by analysis with gas chromatography–mass spectrometry. Detection limits for the ten quinones analyzed are in the range 1–2 nmol dm^{-3}. The potential use of this approach to monitor urinary quinone levels was then evaluated in urine samples from both Sprague-Dawley rats and human subjects. Rats were exposed to 9,10-phenanthraquinone (PQ) by both injection and ingestion (mixed with solid food and dissolved in drinking water). Urinary levels of PQ were found to increase by up to a factor of ten compared to control samples, and the levels were found to depend on both the dose and duration of exposure. Samples were also collected and analyzed periodically from human subjects over the course of six months. Eight quinones were detected in the samples, with levels varying from below the detection limit up to 3 μmol dm^{-3}.

1. Introduction

It is now widely accepted that exposure to high levels of air pollutants such as ozone and particulate matter may lead to adverse health effects. Much of the evidence for health impacts comes from epidemiological studies linking elevated concentrations of various pollutants to increases in mortality and morbidity, e.g. [1–7]. While the evidence for the health effects of air pollution is overwhelming, the mechanisms via which exposure may lead to measurable, clinical symptoms are not well understood and are an active area of research.

Particulate matter with a diameter smaller than 2.5 μm (PM2.5) is one of the criteria pollutants regulated by the US federal government. There is now a large body of literature documenting the impacts of particulate matter (PM) on human health. Air pollution from various sources is an important contributor to morbidity and mortality [8–13], independent of exposure to other pollutants, severe weather or seasonal changes [14–17]. It is generally accepted that exposure to particulate matter may result in oxidative stress that may overwhelm the lung's defense mechanisms, resulting in inflammation and, potentially, a measurable health effect, e.g. [18,19]. The origin of the oxidative stress is still the subject of debate. PM2.5 is a complex mixture of inorganic and organic constituents, and particles are known to contain a range of chemical components that may be involved in initiating oxidative stress. These include metals (such as iron and copper) [20–23] and organics (such as polyaromatic hydrocarbons (PAHs) and quinones) [24–29].

To better understand the relationship between inhalation of PM components, oxidative stress and inflammation, it is necessary to accurately measure exposure to these chemical constituents. Various approaches have been taken to evaluate exposure to environmental pollutants. Predictive models have been developed to assess exposure based on estimated or measured atmospheric pollutant levels, proximity to the pollutant source, and daily behavior of the population [30–34]. When atmospheric levels of pollutants are not routinely monitored (as in the case of quinones), this approach typically requires a number of assumptions to be made that may limit the accuracy of the predicted exposure. This is likely to be amplified in studies with a small sample size, in part due to the potentially large impacts of differences in daily routine that should be averaged out over large sample populations. Personal monitors have also been used to measure exposure of subjects to PM2.5 and some of its chemical constituents. These typically consist of a particle size-selective inlet connected to a sampling pump that is worn by the subject. Personal monitors have been widely used to measure exposure to PM mass, e.g. [35–39], as well as exposure to specific chemical constituents [40–42]. While personal monitors provide an excellent measure of exposure, they are expensive to use with larger sample populations, and since they must be carried during the study period, they are somewhat intrusive for the subjects using them.

An alternative approach to monitor exposure is to use environmental biomarkers. Exposure to pollutants may lead to their uptake into the body, where they are ultimately excreted (either directly or after they have been metabolized). Concentrations of the pollutant

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or its metabolites in biological samples (i.e., blood or urine) have been used to measure the levels of exposure of individuals to these chemicals in previous studies. For example, urinary biomarkers have been developed to measure exposure to wood smoke and PAHs [43–47]. The biomarker approach may provide more accurate exposure data for study subjects than a predictive model, and is more convenient and less expensive than using personal monitors. However, to be successfully used, increases in the biomarker concentration due to exposure must be measurably greater than the natural variability in its levels. Further, differences in response between individual subjects may make interpretation of data more difficult.

In this work, the use of urinary quinone levels as environmental biomarkers for exposure to quinones was explored. A method was developed to measure levels of quinones in urine samples. The potential for the use of this approach was then demonstrated by monitoring urinary quinones in both an animal model and in human subjects.

2. Experimental

2.1. Sample collection

2.1.1. Animal model

Three sets of experiments were carried out using female Sprague-Dawley rats. All procedures were approved by the Institutional Animal Care and Use Committee at California State University, Fresno. Animals were treated according to the Federal Animal Care Guidelines and had free access to rodent chow (LabDiet # 5001; PMI Nutrition International, St. Louis, MO) and water. In the first set of experiments, animals were mildly anaesthetized with ketamine/xylazine, and were then exposed to 0–20 μg 9,10-phenanthraquinone (PQ, ≥99%, Aldrich) or phenanthrene (98%, Aldrich) by injection of 100 μL dimethyl sulfoxide (DMSO, >99.5%, Sigma) solution of the PQ or phenanthrene into the peritoneal cavity. One animal received each dose of PQ or phenanthrene, and two control animals were injected with DMSO. In the second experiment, two animals were fed solid food containing 20 mg PQ kg^{-1}. PQ was again dissolved in DMSO and adsorbed to the surface of regular rodent chow. In the final experiment, five animals were given drinking water containing 10 μg mL^{-1} of PQ (PQ/DMSO solution was dissolved in regular drinking water). A control group of six animals were provided with quinone-free water and regular chow, but were otherwise housed under identical conditions.

Urine samples were collected from each animal at a series of time points following the initial exposure. The animals were mildly anaesthetized with intramuscular ketamine/xylazine injections and approximately 2 mL of urine was collected from each animal in a plastic bag (animals will spontaneously void their bladder as they regain consciousness). Samples were transferred to vials and were stored at −70 °C until analyzed. Quinones were then extracted from the samples and analyzed as described below.

2.1.2. Human samples

The work involving human subjects followed procedures approved by the Institutional Review Board at California State University, Fresno. Urine samples were periodically collected from two human subjects over the course of several months. The volunteers for these measurements were healthy adult non-smokers. The samples were systematically collected in the morning and were frozen until analyzed, which typically occurred within 24 h of collection.

2.1.3. Sample preparation

Rat urine samples were first spiked with anthraquinone (97%, Aldrich), which was used as an internal standard. All samples (2 mL rat urine or 10 mL human urine) were mixed with 2 mL of saturated potassium carbonate (ACS Reagent Grade, Sigma–Aldrich) solution and 2 mL of dichloromethane (>99.9%, Sigma–Aldrich). The mixture was shaken for 5 min, then centrifuged for 10 min following which the organic layer was removed from the mixture. A 0.5 mL aliquot of the dichloromethane layer was used for direct analysis by gas chromatography–mass spectrometry (GC–MS). Quinones present within a second 0.5 mL aliquot were converted to their diacetyl derivatives prior to analysis. Previous work has demonstrated that this approach can improve the detection limit for several quinones, including PQ [48,49]. The 0.5 mL dichloromethane extract was mixed with 0.2 mL acetic anhydride (>99%, Sigma–Aldrich) and 0.1 g zinc powder (>99.9%, Sigma–Aldrich). The mixture was shaken and then heated to 80 °C for 15 min in a dry bath incubator. The samples were removed every 5 min and shaken. The samples were then allowed to cool to room temperature before an additional 0.1 g zinc was added, and the mixture was again heated to 80 °C for 15 min, and shaken at 5 min intervals. The samples were cooled and 0.5 mL deionized water was added, followed by 3 mL pentane (>99%, Sigma–Aldrich). The contents were again shaken and centrifuged and the pentane layer was removed. In preliminary samples, the pentane layer was evaporated to dryness and reconstituted in 0.5 mL dichloromethane. However, most of the samples were analyzed in the pentane layer following centrifuging due to the uncertainties introduced in the last evaporation/reconstitution step (see below).

2.2. Sample analysis

All sample extracts were analyzed by GC–MS (GC–MS, HP6890, Agilent Technologies) as described in previous work [49]. Underivatized quinones (acenaphthenequinone, >97%, Fluka; anthraquinone; 2-methyl anthraquinone, >95%, Aldrich; 2,3-dimethyl anthraquinone, >95%, Aldrich; 5,12-naphthacenequinone, 97%, Aldrich; and benz[a]anthracene-7,12-dione, 98%, Aldrich) and derivatized quinones (2,6-di-*tert*-butyl-1,4-benzoquinone, 98%, Aldrich; 1,2-naphthoquinone, 97%, Aldrich; 1,4-naphthoquinone, 97%, Aldrich; 9,10-phenanthraquinone; and 1,4-chrysenequinone) were quantified in separate chromatographic runs. For both methods, 5 μL of sample was injected into the heated inlet in the splitless mode. Samples were separated on an HP-5MS column (30 m × 0.15 mm × 0.37 μm film thickness, Agilent Technologies). The column was held at 100 °C for 4 min and was then ramped at 5 °C min^{-1} to 310 °C and held for 5 min for a total run time of 51 min. Analytes were identified and quantified in the selected ion monitoring (SIM) mode by comparison to mixtures of authentic standards prepared in the laboratory using the same *m*/*z* ratios as in previous measurements, as shown in Table 1.

Calibration standards (1×10^{-8}–1×10^{-4} mol dm^{-3}) were generated by spiking known quantities of each chemical species into authentic human urine samples. Urine samples were analyzed prior to spiking to ensure that they did not contain measurable levels of the target compounds. The calibration standards were then extracted and analyzed as described above. Fresh standards were made immediately before analysis since the quinone signals were found to decrease significantly when stored for 24 h or longer.

3. Results and discussion

3.1. Method validation

The protocol for the extraction and analysis of urinary quinones was developed as part of this work. A series of experiments was therefore performed to evaluate the reproducibility and detection limits of the method. The original method consists of four individual

Table 1
Parameters for the analysis of quinones by gas chromatography–mass spectrometry.

Quinone	Monitored ions	Retention time (min)	Limit of quantitation (mol dm^{-3})
Acenaphthenequinone	126, 154, 182[a]	22.4	1×10^{-9}
Anthraquinone	152, 180, 208[a]	24.2	2×10^{-9}
2-Methyl anthraquinone	168, 196, 222[a]	26.6	2×10^{-9}
2,3-Dimethyl anthraquinone	180, 208, 236[a]	29.8	1×10^{-9}
5,12-Naphthacenequinone/benz[a]anthracene-7,12-dione[b]	202, 230, 258[a]	35.0	1×10^{-9}
2,6-di-*tert*-Butyl-1,4-benzoquinone[c]	222[a], 264, 306	20.5	2×10^{-9}
1,2-Naphthoquinone[c]	160[a], 202, 244	22.7	1×10^{-9}
1,4-Naphthoquinone[c]	160[a], 202, 244	24.1	1×10^{-9}
9,10-Phenanthraquinone[c]	210[a], 252, 294	33.1	1×10^{-9}
1,4-Chrysenequinone[c]	260[a], 302, 244	43.3	1×10^{-9}

[a] m/z used for quantitation.
[b] Quinones cannot be resolved with the method used.
[c] Quinones are measured as the diacetylated derivatives (see text for details).

steps: extraction, derivatization (for certain quinones), concentration and analysis. Calibration standards of 1×10^{-6} mol dm^{-3} PQ in human urine were divided into five samples and taken through the entire procedure. The standard deviation of the average of these five analyses was 29%. Experiments were then carried out to determine the contribution of each step to the overall uncertainty.

3.1.1. Instrument response

The signal response for five replicate analyses of a 1×10^{-6} mol dm^{-3} phenanthraquinone solution in dichloromethane was determined. The standard deviation of the signal response was less than 1%.

3.1.2. Extraction

The extraction efficiency for the quinones was tested both with and without the addition of saturated potassium carbonate to the urine samples. A 50 mL sample of urine was spiked with phenanthraquinone to make a 1×10^{-6} mol dm^{-3} solution, and was separated into five 10 mL samples. Potassium carbonate was added to each sample, and was subsequently extracted with two 2 mL aliquots of dichloromethane. The phenanthraquinone concentration in each extract was then analyzed by GC–MS as described above. This procedure was then repeated, but without the potassium carbonate addition step. For all samples containing potassium carbonate, the second extract did not contain measurable levels of phenanthraquinone. The standard deviation for the average signal from the five extracts was determined to be 1%. When the potassium carbonate was not added during the extraction, phenanthraquinone was observed in the second extracts with a signal about 20% of the first extract.

3.1.3. Derivatization

Five 2 mL samples of 1×10^{-6} mol dm^{-3} phenanthraquinone solution were derivatized according to the procedure described above and then analyzed by GC–MS. The standard deviation of the signal from the samples was determined to be 13%.

3.1.4. Concentration

Five 2 mL samples of a 1×10^{-6} mol dm^{-3} phenanthraquinone standard in dichloromethane were evaporated to dryness under a stream of dry air, and were redissolved in 0.5 mL of dichloromethane. The standard deviation of the signal response was found to be 29%.

These measurements show that the concentration step is the largest individual contributor to the overall uncertainty. Since this step does not result in a substantial improvement in detection limits, this procedure was removed from the method and samples were analyzed immediately following the extraction step in the pentane layer. The overall uncertainty in the procedure used to analyze rat and human urine samples is 15%. The limits of detection and quantification were taken to be the concentrations corresponding to signals three and six times greater than the noise at the appropriate retention time. Values for the species monitored are listed in Table 1.

3.2. Animal model

Urinary PQ levels were measured in samples collected from female Sprague-Dawley rats exposed via injection and ingestion (food and water). In each case, the PQ signal was normalized to the signal from the anthraquinone internal standard. Urinary signals are affected by, among other factors, the concentration of the urine, animal size and the rate at which the analyte is metabolized. Here we assume that ketamine, which is used to anaesthetize the animals, may be metabolized/excreted by the animals on a similar timescale to PQ, and so the quinone signals were also normalized to the urinary ketamine concentration.

3.2.1. Exposure by injection

Urine samples were collected from animals 24 h after injection of PQ, phenanthrene or pure DMSO. Measurable levels of PQ were observed in all of the samples, including the pure DMSO group. Normalized PQ signals are shown in Fig. 1 for animals injected with 0, 20 ng, 200 ng, 2 μg and 20 μg of PQ and phenanthrene in DMSO. The urinary PQ levels for all animals exposed to either PQ or phenanthrene is substantially higher than the levels in animals injected with DMSO only. A general increase in the urinary PQ signal was observed as the amount of PQ injected increased. No relationship between the phenanthrene dose and the PQ signal is apparent.

Ambient levels of quinones are often in the range of 1–10 ng m^{-3} during particulate matter air pollution episodes in Central California. This translates into daily exposures on the order of 10^1–10^2 ng day^{-1} during these periods, which falls within the range of exposures tested here. However, the lower relative body mass of the animals results in a significantly higher exposure (dose per kilogram of body weight) for the animals compared to the predicted human inhalation exposure described above.

It is well established that PAHs are metabolized within the body to generate a range of products including quinones. It is therefore not surprising that exposure to phenanthrene results in elevated urinary levels of PQ. The atmospheric concentrations of PAHs may exceed the levels of the corresponding quinone by a factor of ten

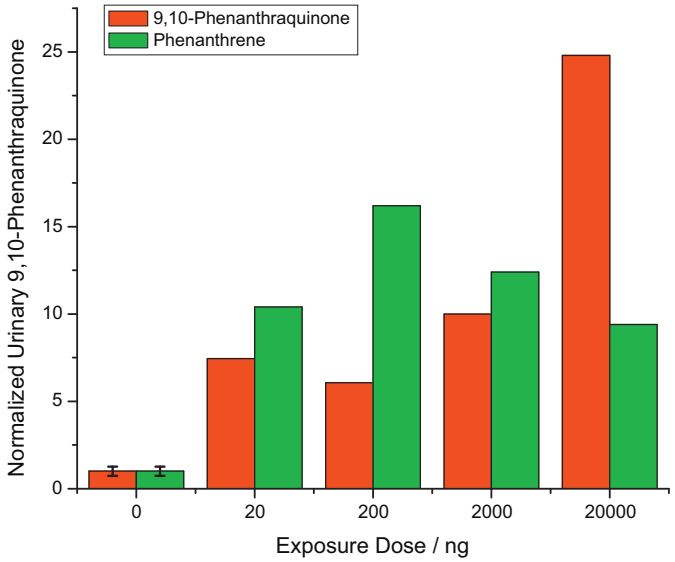

Fig. 1. Relative 9,10-phenanthraquinone concentrations in rat urine collected 24 h after injection with 9,10-phenanthraquinone, phenanthrene or dimethylsulfoxide. Quinone signals are shown relative to the average signal from the control samples. (For interpretation of the references to color in this figure, the reader is referred to the web version of the article.)

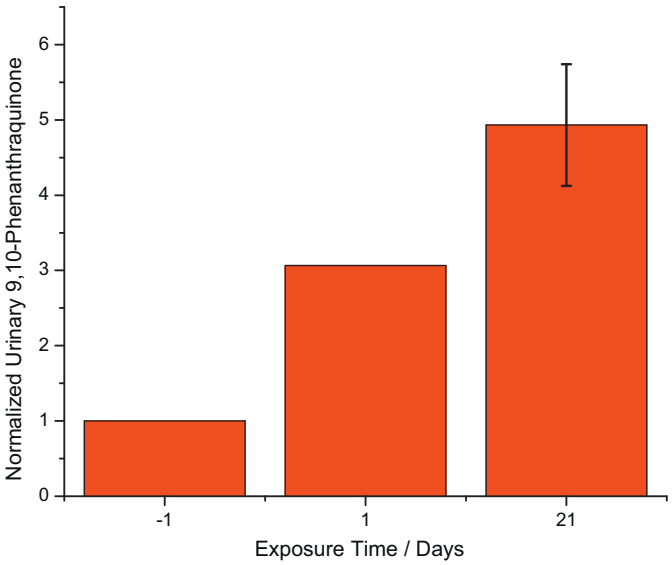

Fig. 2. Relative urinary 9,10-phenanthraquinone concentrations in rat urine after exposure to 9,10-phenanthraquinone in solid food. Animals were fed with 20 mg phenanthraquinone per kg solid food beginning on Day 0. Quinone signals are shown relative to the signal measured before exposure began (Day −1).

or more. Since exposure to both PQ and phenanthrene result in elevated levels of urinary PQ, this may be a biomarker for both compounds.

In these experiments, the limited availability of animals meant that with the exception of the control animals ($N = 2$), no replicates could be performed. Given the potential biological variability between animals, some scatter in the dose–response relationship is to be expected. The pathways leading from injection to excretion are likely to be significantly more complex for phenanthrene compared to PQ since the PAH must be metabolized to the quinone. This may at least partially explain the lack of correlation between the PAH dose and the urinary PQ signal.

The amount of PQ recovered in the urine following injection of 20 ng PQ is approximately 80% based on the urinary concentration and the volume collected. It seems very unlikely that such a high recovery would be obtained in a single urine sample, suggesting some of the observed PQ urinary signal may be from a different source. Since the analytical procedure for PQ is very specific for this compound, the probability that the signal is from a co-eluting peak with a similar mass spectrum is low. It is possible that some of the observed signal is 'background' PQ that is naturally present in the animals in the absence of the injected PQ. However, the difference between the exposed animals and the control animals is statistically significant at the 95% confidence limit ($P = 0.001$), consistent with a measurable effect resulting from the exposure of the experimental group. Another possibility is that exposure to phenanthrene and PQ activates a biological pathway leading to an increase in urinary PQ, effectively amplifying the observed signals. However, given the limited amount of data available, no clear conclusions can be drawn.

3.2.2. Exposure in solid food

The urinary PQ levels from an animal fed PQ in its solid food beginning on Day 0 are shown for Day −1 (i.e., one day prior to PQ exposure), Day 1 and Day 21 are shown in Fig. 2. One day after being fed PQ, the level of urinary PQ rose by a factor of three from the pre-exposure level. After three weeks of exposure, the signal was approximately five times higher than the pre-exposure level. The measured increase in urinary PQ within 24 h of exposure is consistent with the results of the exposure-by-injection experiments described above. The data also indicate that prolonged exposure to PQ has a cumulative effect that may lead to higher urinary PQ levels compared to short-term exposure.

3.2.3. Exposure in drinking water

PQ levels measured in urinary samples collected from animals exposed to PQ in their drinking water are shown in Fig. 3. A systematic increase in signal is observed as the exposure time increases, with an average urinary PQ level that is two and five times higher than in the control group after two and three weeks of exposure, respectively. The average signal after three weeks of exposure is significantly higher than the signal in the control group ($P = 0.04$),

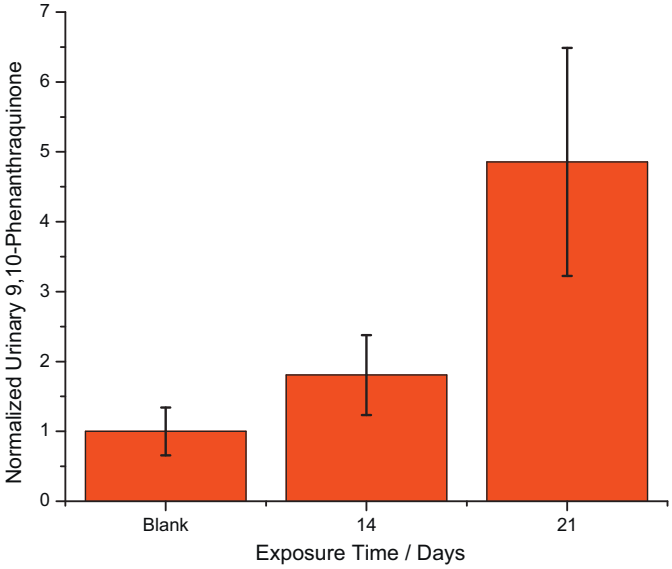

Fig. 3. Relative urinary 9,10-phenanthraquinone concentrations in rat urine after exposure 9,10-phenanthraquinone in drinking water. Animals received 10 μg phenanthraquinone per mL drinking water beginning on Day 0. Quinone signals are shown relative to the signal measured from controls exposed to quinone-free water.

Table 2
Urinary quinone concentrations in human subjects.

Quinone	Subject 1[a] ($N = 11$)			Subject 2 ($N = 8$)		
	Mean concentration (mol dm^{-3})	Standard deviation of the mean (mol dm^{-3})	Maximum concentration (mol dm^{-3})	Mean concentration (mol dm^{-3})	Standard deviation of the mean (mol dm^{-3})	Maximum concentration (mol dm^{-3})
Acenaphthenequinone	3.6×10^{-9}	3.2×10^{-9}	3.4×10^{-8}	8.6×10^{-10}	6.8×10^{-10}	5.6×10^{-9}
Anthraquinone	7.0×10^{-8}	1.8×10^{-7}	3.8×10^{-7}	1.4×10^{-7}	1.1×10^{-7}	8.6×10^{-7}
2-Methyl anthraquinone	1.3×10^{-9}	8.4×10^{-10}	9.4×10^{-9}	5.6×10^{-9}	5.0×10^{-9}	4.0×10^{-8}
5,12-Naphthacenequinone/benz[a] anthracene-7,12-dione	8.1×10^{-9}	1.0×10^{-9}	1.0×10^{-8}	8.2×10^{-10}	5.4×10^{-10}	3.4×10^{-9}
1,4-Naphthoquinone	1.1×10^{-8}	4.2×10^{-9}	5.1×10^{-8}	3.9×10^{-9}	1.7×10^{-9}	1.5×10^{-8}
9,10-Phenanthrenequinone	2.0×10^{-7}	1.1×10^{-7}	1.3×10^{-6}	2.5×10^{-8}	1.8×10^{-8}	1.4×10^{-7}
2,6-di-*tert*-Butyl-1,4-benzoquinone	4.8×10^{-8}	1.9×10^{-8}	1.9×10^{-7}	4.2×10^{-9}	2.3×10^{-9}	1.7×10^{-8}
1,4-Chrysenequinone	2.3×10^{-7}	7.2×10^{-8}	6.3×10^{-7}	1.4×10^{-8}	1.4×10^{-8}	1.1×10^{-7}

[a] Excludes outliers on 04.20.07.

but the difference between the two-week exposure signal and both the control group and the three week exposure signals are not statistically significant ($P = 0.13$ and $P = 0.07$, respectively).

3.3. Human subject samples

To evaluate the potential for this approach to be used to monitor urinary quinones in human subjects, samples were collected and analyzed periodically from two volunteers over a five month period. Eight quinones (acenaphthenequinone, anthraquinone, 2-methyl anthraquinone, 5,12-naphthacenequinone/benz[a]anthracene-7,12-dione, 1,4-naphthoquinone, PQ, 2,6-ditert-butyl-1,4-benzoquinone and 1,4-chrysenequinone) were observed in the human urinary samples at levels above the detection limit. Measured urinary quinone concentrations ranged from below the limit of detection up to the maximum concentrations given in Table 2. Average urinary quinone concentrations for both subjects are also given in Table 2. Relative concentrations of the three most prevalent quinones (PQ, 1,4-naphthoquinone and chrysenequinone) are also shown in Fig. 4. In this representation, the concentrations of each quinone in each subject are shown as a percentage relative to the highest concentration observed for the analyte from the subject during the study period. For both subjects, quinone signals vary over several orders of magnitude. The standard deviation of the mean concentrations of

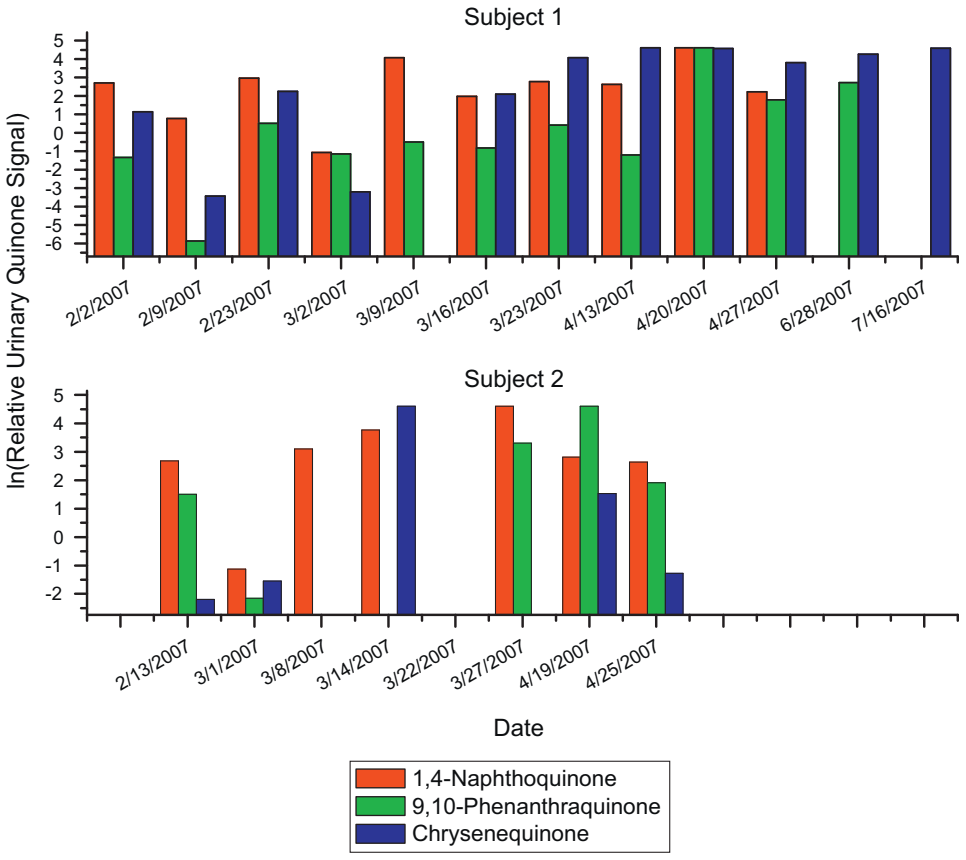

Fig. 4. Relative urinary concentrations of 1,4-naphthoquinone, 9,10-phenanthraquinone and chrysenequinone in human subjects. The concentrations for each quinone in each subject are normalized to the highest concentration observed during the study period. (For interpretation of the references to color in this figure, the reader is referred to the web version of the article.)

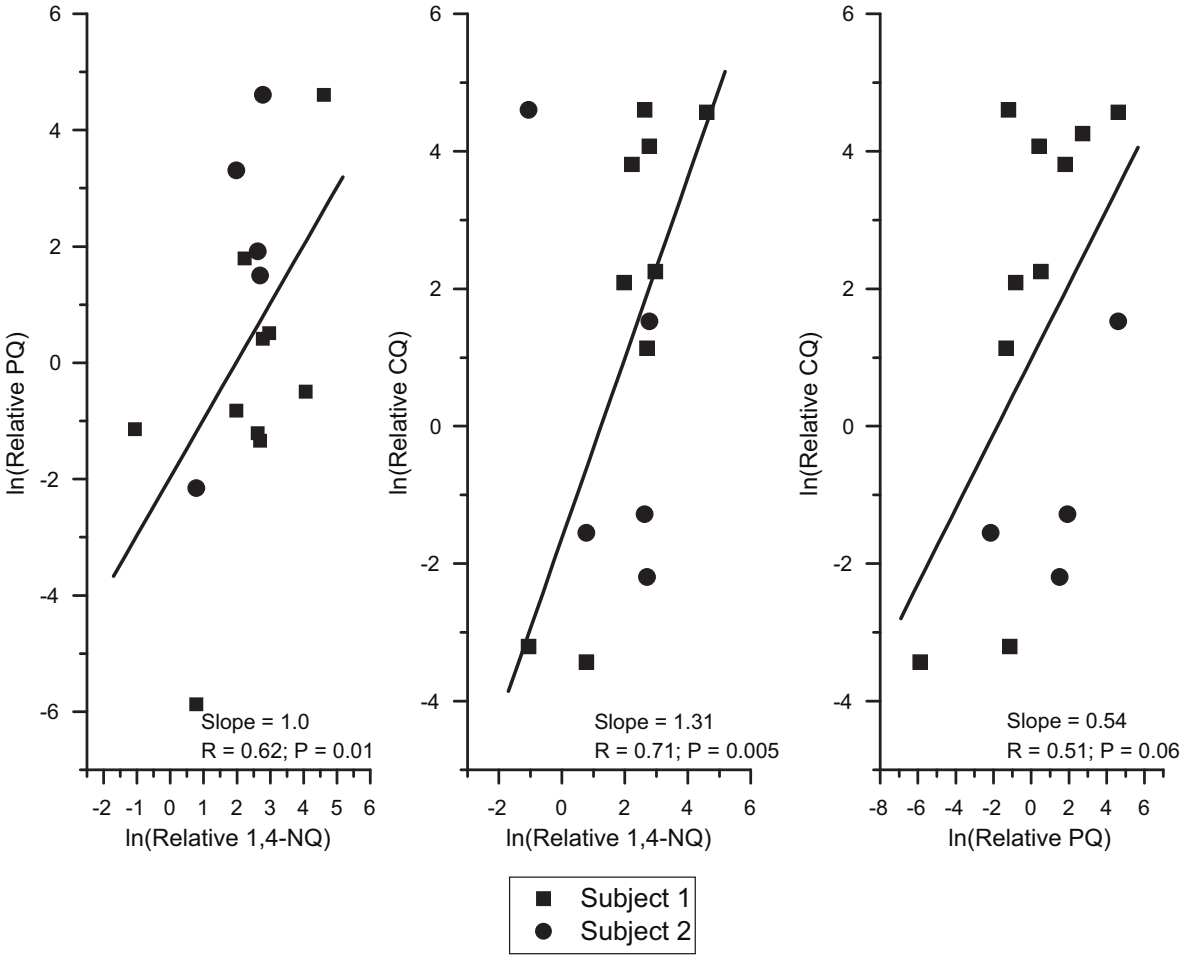

Fig. 5. Relationship between the relative urinary concentrations of 1,4-naphthoquinone (1,4-NQ), 9,10-phenanthraquinone (PQ) and chrysenequinone (CQ) in human subjects. The concentrations for each quinone in each subject are normalized to the highest concentration observed during the study period.

PQ, 1,4-naphthoquinone and chrysenequinone are in the range 10^{-7}–10^{-9} mol dm^{-3} for Subject 1, and 10^{-8}–10^{-9} mol dm^{-3} for Subject 2.

A urinary concentration of 1×10^{-8} mol dm^{-3} PQ corresponds approximately to a dose of 3 μg day^{-1}, assuming a total excreted volume of urine of 1.5 L day^{-1}. If inhalation were the only source of the measured urinary quinones, and given that an adult will inhale air on the order of 15 m^3 day^{-1}, this would translate into a mass loading of about 200 ng m^{-3}. Maximum PQ mass loadings in Fresno are less than 10 ng m^{-3} [49] and phenanthrene concentrations are typically below 100 ng m^{-3} (e.g. [50]). This implies that exposure to typical ambient concentrations of PQ and phenanthrene cannot be solely responsible for observed variations in urinary PQ. Of the quinones and parent PAHs of the quinones observed in the human urine samples, only naphthalene is typically present at high enough atmospheric concentrations to potentially be responsible for the observed urinary levels [50]. However, exposure to sources of ambient PAHs and quinones, such as diesel exhaust [51,52] and cigarette smoke [53], may result in exposures that are much higher than typical atmospheric concentrations. PAHs are known to be present in fresh and processed foods [54]. For most PAHs, ingestion may be a substantially larger source of exposure than inhalation. Since PAHs and quinones likely share similar sources, diet may also play a role in exposure to quinones, and is expected to be a factor that influences urinary quinone levels.

Since the samples from the two subjects were collected on different days, they cannot be compared directly. However, the relative concentrations of the three most prevalent quinones are reasonably well correlated with each other. Plots of the log-transformed relative quinone signals (plotted in Fig. 4) are shown in Fig. 5. The relationships between PQ and 1,4-naphthoquinone ($r = 0.62$; $P = 0.01$) and between chrysenequinone and 1,4-naphthoquinone ($r = 0.71$; $P = 0.005$) are statistically significant, while the correlation between chrysenequinone and PQ ($r = 0.51$; $P = 0.06$) is approaching statistical significance. These data suggest that the three quinones originate from the same environmental sources or that they are generated via related biological pathways. However, in the absence of additional information it is not possible to determine if the levels of these urinary quinones are linked to environmental exposure.

4. Conclusions

The analytical approach developed in this work has been shown to be capable of detecting and quantifying quinones in urine samples at naturally occurring levels. The experiments carried out in a rat model provide strong evidence that urinary quinone levels are correlated with environmental exposure to both the quinones themselves and the parent PAHs. However, the exposure doses used in these experiments are significantly higher (dose per kilogram of body weight) compared to those that are expected to result from human exposure to ambient particulate matter. The measurements show that urinary levels of several quinones can be routinely monitored using the method developed in this work. However, additional measurements are required to determine whether urinary quinones can be used as biomarkers for

exposure to atmospheric or other environmental sources of PAHs and quinones.

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