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Oxidative stress and respiratory symptoms due to human exposure to polycyclic aromatic hydrocarbons (PAHs) in Kumasi, Ghana

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Abstract

Studies of polycyclic aromatic hydrocarbons (PAHs) and its metabolites in PM10, soils, rat livers and cattle urine in Kumasi, Ghana, revealed high concentrations and cancer potency. In addition, WHO and IARC have reported an increase in cancer incidence and respiratory diseases in Ghana. Human urine were therefore collected from urban and control sites to: assess the health effects associated with PAHs exposure using malondialdehyde (MDA) and 8-hydroxy-2-deoxyguanosine (8-OHdG); identify any association between OH-PAHs, MDA, 8-OHdG with age and sex; and determine the relationship between PAHs exposure and occurrence of respiratory diseases. From the results, urinary concentrations of the sum of OH-PAHs (ΣOHPAHs) were significantly higher from urban sites compared to the control site. Geometric mean concentrations adjusted by specific gravity, GM_{SG}, indicated 2-OHNaphthalene (2-OHNap) (6.01 ± 4.21 ng/mL) as the most abundant OH-PAH, and exposure could be through the use of naphthalene-containing-mothballs in drinking water purification, insect repellent, freshener in clothes and/or “treatment of various ailments”. The study revealed that exposure to naphthalene significantly increases the occurrence of persistent cough (OR = 2.68, CI: 1.43-5.05), persistent headache (OR = 1.82, CI: 1.02-3.26), tachycardia (OR = 3.36, CI: 1.39-8.10) and dyspnea (OR = 3.07, CI: 1.27-7.43) in Kumasi residents. Highest level of urinary 2-OHNap (224 ng/mL) was detected in a female, who reported symptoms of persistent cough, headache, tachycardia, nasal congestion and inflammation, all of which are symptoms of naphthalene exposure according to USEPA. The ΣOHPAHs, 2-OHNap, 2-3-OHFluorenes, and -OHPhenanthrenes showed a significantly positive correlation with MDA and 4-OHPhenanthrene with 8-OHdG,
indicating possible lipid peroxidation/cell damage or degenerative disease in some participants. MDA and 8-OHdG were highest in age group 21-60. The present study showed a significant sex difference with higher levels of urinary OH-PAHs in females than males.

**Keywords:** OH-PAHs; Kumasi; Odds; Human; Urine

**Capsule:** In addition to lipid peroxidation/cell damage, PAHs exposure increased occurrence of respiratory diseases in residents of Kumasi, Ghana.

1. **Introduction**

Polycyclic aromatic hydrocarbons (PAHs) are the 9th most hazardous substance (ATSDR 2015) and formed during combustion of organic materials such as fuel, wood or grass. They are also found in cigarette smoke and grilled foods. Human and animal exposure to PAHs occur mainly through inhalation of contaminated air or ingestion of soil, food and/or drinking contaminated water (Barranco et al. 2004). In humans and animals, PAHs are metabolized by cytochrome P450 enzymes to generate active semiquinones (Penning et al. 2007) which are free radical intermediates and can go through redox cycling and generate reactive oxygen species (ROS). The ROS can then cause oxidative modification of DNA and lipids in the body (Palackal et al. 2002). The DNA adduct, 8-hydroxy-2’-deoxyguanosine (8-OHdG), is a useful biomarker of DNA damage to assess human exposure to carcinogenic compounds and malondialdehyde (MDA) is an indicator for oxidative stress and lipid peroxidation (Basu and Marnett 1983).
Epidemiological studies have reported associations between PAHs exposure and urinary MDA and 8-OHdG in children, workers, as well as general population in several countries (Fan et al. 2012a; Kuang et al. 2013; Lee et al. 2012). 8-OHdG is very stable, excreted in urine without being metabolized (Valavanidis et al. 2009) and measurement could offer a specific and quantitative biomarker indicating oxidative DNA damage caused by ROS which is a risk factor for cancer and cardiovascular disease (Shen and Ong 2000). Exposure to PAHs have also been associated with adverse respiratory health outcomes, increased cough and wheeze, decreased lung function, bronchitis, nasal symptoms, breathing problems as well as development of asthma in both children and adults (USEPA 1994; Al-Daghri et al. 2013; Liu et al. 2016).

The growing rate of industrialization, economy and population has led to contamination and deterioration of the environment and pollution is likely to reach disturbing levels in Kumasi, Ghana (Bortey-Sam et al. 2014). Studies by Bortey-Sam et al. (2013, 2014, 2015) in particulate matter (PM10) and soils indicated that the city centre of Kumasi has been polluted with PAHs when compared with recommended levels (Directive 2004/107/EC; WHO 2000). The total benzo(a)pyrene equivalent concentration and estimated carcinogenicity of PAHs in PM10 and soil from the city centre was approximately 18 and 150 times higher, respectively, as compared to a pristine site. Again, in the city centre of Kumasi, high concentrations of PAHs were detected in the livers of wild rats and naphthalene was detected in 80% of livers (Bortey-Sam et al. 2015b). Cattle urine was further used as a “biological indicator” to assess human risk in Kumasi and high levels of
urinary OH-PAHs were detected and 2-hydroxy naphthalene was most abundant (Bortey-Sam et al. 2016).

The number of cancer cases in Ghana has drastically increased (WHO 2010, 2011) and GLOBOCAN estimated 16,600 cases of cancer annually in Ghana, yielding an age standardized rate of 109.5 cases per 100,000 people (IARC GLOBOCAN 2008). In addition, the occurrence of respiratory diseases doubled in Ghana from 2004-2008, and 5728 deaths were attributed to ambient air pollution in 2012 (WHO 2010, 2011, 2016); and PAHs could play a significant role. The commonest sites for cancers reported among both sexes in Kumasi were breast (24%), cervix uteri (20%), ovary (7.9%), liver (6.4%) and prostate (4.0%) (Laryea et al. 2014). PAHs have been shown to accumulate in breast tissues and led to breast cancer in humans (Korsh et al. 2015; Mordukhovich et al. 2016; White et al. 2016). The estimated cancer incidence for 2012 in Kumasi was 11.9 per 100,000. Among males and females, the cancer incidences were 7.3 and 15.7 per 100,000, respectively (Laryea et al. 2014).

With the high cancer incidence and toxic potential of PAHs and its metabolites in Kumasi (Bortey-Sam et al. 2014, 2015, 2016, Laryea et al. 2014), and due to lack of epidemiological studies on the risks of PAHs exposure to Kumasi residents, the objectives of the present study were to: assess the health effects due to PAHs exposure to residents of Kumasi; find the association between urinary concentrations of OH-PAHs, MDA, 8-OHdG with age and sex.

2. Materials and methods

2.1. Sampling
In January to February 2015, human urine samples (n = 190: 57 males and 133 females; including 16 urine samples collected from 3-12-year-old children) were collected in the morning from the general population of Atonsu, Manhyia and Tafo communities in Kumasi. The urine samples were collected into labelled corning tubes (Corning Incorporated, New York, USA). Manhyia is 1.1, 1.2 and 1.5 km away from Kejetia, Romanhill and Adum (city centre), respectively, which were polluted with PAHs (PM10 and soils) and also recorded the highest sum of PAHs in livers of wild rats (Bortey-Sam et al. 2014, 2015, 2015b). Tafo is 2.3 and 2.6 km from Suame and Mbrom, respectively, whose soils were polluted with PAHs (Bortey-Sam et al. 2014) (Fig. 1). Urine was collected from willing participants of both sexes and all ages. Residents of other regions were not included in the study except those who had lived in the study area for over three years. Variations in temperature both annually and daily are quite small and the minimum temperature is around 23 °C (73 °F) (Bortey-Sam et al. 2015). The coldest/wettest season is June to September.

Due to the lack of background urine and interferences during OH-PAHs quantification, 400 mL of human (children) urine (blank stock) was collected from 4 participants in residential areas of the Kwame Nkrumah University of Science and Technology (KNUST) campus to form a composite. KNUST is a public university located in Kumasi, Ghana, and because of the low vehicular movement, industrial and human activities, PAHs exposure from point sources were assumed to be negligible (Bortey-Sam et al. 2015). In addition, PM10 and soils at KNUST were used as reference/control for PAHs studies (Bortey-Sam et al. 2013; 2014; 2015). However, because humans could be exposed to PAHs through feed and/or inhalation, the sample collected was measured several times to confirm levels of
OH-PAHs. Additionally, 12 human urine (7 males and 5 females; 21-46 years) were collected from KNUST campus for comparison (reference samples) although there could be PAH exposure through feed and/or inhalation.

Participant’s information, including age, gender, body weight, height, place of residence, occupation, and personal lifestyle including smoker/non-smoker, were obtained through face-to-face interviews. In addition, the presence or absence of symptoms of PAHs exposure such as persistent cough, persistent headache, asthma, wheeze, respiratory tract infection (RTI), tachycardia, rhinitis/nasal congestion or inflammation and dyspnea (USEPA 1994; Al-Daghri et al. 2013; Liu et al. 2016) were collected. The study was performed in accordance with the guidelines and approval of the Ethical/Institutional Review Board of Ghana Health Service (GHS) and Council for Scientific and Industrial Research (CSIR), Accra, Ghana, after informed consent was obtained from each participant. Parent(s) gave written or informed consent and completed questionnaires on behalf of their child/children. Samples collected were stored at –20 °C in the Department of Chemistry, KNUST, Ghana and later transported to the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan, where they were stored at –30 °C until analysis.

2.2. Sample extraction and analysis

2.2.1. OH-PAHs

Extraction of OH-PAHs from human urine was done according to method described by Bortey-Sam et al. (2016) with slight modification. Briefly, 20 µL each of β-glucuronidase (bovine liver, type B-1; 1240 U/mg; Sigma Aldrich) and arylsulfatase (limpets Type V; 34
units/mg; Sigma Aldrich) enzymes, and 5 mL of 0.1 M sodium acetate buffer (pH 5.6) were added to 5 mL urine sample after spiking with three PAH internal standards (13C6-2-OHFluorene, 3-OHPhenanthrene-d9, and 13C6-1-OHPyrene). The pH of sample was adjusted to 5.5 using 1 M acetic acid (Wako Pure Chemicals, Osaka, Japan) and incubated overnight at 37 °C. The sample was diluted with 4 mL of Milli-Q water and extracted twice (liquid-liquid extraction) with 10 mL each of n-pentane (Kanto Chemical Corp., Tokyo, Japan) by shaking for 1 h. To reduce the interference of sulfur metabolites, the combined extracts were washed with 2 mL of 1 N AgNO3 solution (Wako Pure Chemicals, Osaka, Japan), concentrated to 50-100 µL, re-dissolved to 0.5 mL using methanol and filtered (0.20 µm DISMIC-13JP membrane filter, ADVANTEC, Toyo Roshi Kaisha Ltd., Japan) prior to instrumental analysis. All sample preparation steps were performed in darkness (by covering tubes completely with aluminium foil) to avoid possible photodegradation of target analytes. A total of 13 OH-PAHs; 2-hydroxynaphthalene (2-OHNap), 2-hydroxyfluorene (2-OHFlu), 3-hydroxyfluorene (3-OHFlu), 9-hydroxyfluorene (9-OHFlu), 1-hydroxyphenanthrene (1-OHPhe), 2-hydroxyphenanthrene (2-OHPhe), 3-hydroxyphenanthrene (3-OHPhe), 4-hydroxyphenanthrene (4-OHPhe), 9-hydroxyphenanthrene (9-OHPhe), 1-hydroxypyrene (1-OHPyr), 6-hydroxychrysene (6-OHChry), 3-hydroxybenzo(e)pyrene (3-OHBeP) and 9-hydroxybenzo(a)pyrene (9-OHBaP), were analyzed in each sample. The standards (purity ≥ 98%) were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA) and Toronto Research Chemical Inc. (Brisbane Road, North York, Canada). Difficulties were often associated with the separation of 1-OHPhe and 9-OHPhe, for this reason, the sum of these isomers
was used as an abbreviation, 1-9-OHPhe. Similarly, 2-3-OHFlu was used as sum of 2-OHFlu and 3-OHFlu. All results were adjusted by specific gravity and expressed in ng/mL.

A Shimadzu 8030 triple quadrupole mass spectrometer, upgraded to 8040 with UF lens, (ESI MS-MS; Shimadzu, Kyoto, Japan), equipped with a Prominence UFLC system (Shimadzu, Kyoto, Japan) was used for analysis. Chromatographic separation was achieved using an Agilent Eclipse PAH column (150 mm × 2.1 mm, 3.5 μm). The mobile phases were methanol:water (2:3, v:v) (A) and methanol (B), pumped at a flow rate of 250 μL/min. The mobile phase gradient was maintained as follows: 0.0–2.0 min, 5% B; 2.0–20 min, 40% B; 20–25 min, 40% B; 25–30 min, 95% B; 30–35 min, 95% B; 35–35.01 min, 5% B. Target compounds were determined by multiple-reaction monitoring (MRM) in the negative ionization mode (Bortey-Sam et al. 2016).

2.2.2. Malondialdehyde, MDA (Elisa kit)

Concentrations of urinary MDA were measured according to the manufacturer’s instructions using a UV-VIS Spectrophotomer (UV-2600 Shimadzu Corporation, Kyoto, Japan). Briefly, 250 uL of calibrator (0, 1, 2, 3 and 4 μM) or urine sample was added to 10 uL of butylated hydroxytoluene (BHT) reagent in a vial. 250 uL each of acid reagent (1 M phosphoric acid) and 2-thiobarbituric acid (TBA) reagent were added and the mixture was vortexed vigorously then incubated at 60 °C for 1 hr. After centrifugation at 10000 x g for 2-3 min, the reaction mixture was transferred into a cuvette and spectra was recorded from 400-700 nm. 3rd derivative analysis was performed at 514 nm.

2.2.3. 8-hydroxy-2-deoxy-guanosine (8-OHdG)
1 mL aliquot of each urine sample was diluted with 2 mL of water (HPLC grade) after spiking with 25 ng/mL of the stable isotope labelled internal standard, (15N5) 8-OHdG. The diluted sample was subjected to solid-phase extraction using Oasis HLB cartridge (3cc, 60 mg; Waters Corporation, Milford, MA, USA) that had been primed with 1 mL each of methanol and water. After sample loading, the cartridge was sequentially washed with 3 mL of water. 8-OHdG was eluted with 3 mL of water: acetonitrile (1:1, v/v), and evaporated to near dryness under nitrogen gas. The residue was re-dissolved in 100 µL of water, and 10 µL was injected into the LC–MS/MS. Chromatographic separation of 8-OHdG and (15N5) 8-OHdG in urine were performed using a Phenomenex Gemini 3u C18 110A column (150 mm × 2 mm i.d., 4 micron, Phenomenex, California, USA) with a guard column. Gradient elution was as follows: 0.0–1.0 min, 5% B; 1.01–3.00 min, 50% B; 3.01–6.00 min, 5% B. Target compounds were determined by MRM in negative ionization mode at a column temperature of 40 °C. Mobile phases A (0.1% formic acid) and B (100% methanol) were pumped at a flow rate of 250 µL/min.

2.3. Specific gravity (SG) of human urine

To compensate for variations in urine dilution, urinary OH-PAH, MDA and 8-OHdG concentrations were adjusted by specific gravity (SG). In this study SG was used because the use of creatinine (CR) to normalize urinary analyte concentrations could be problematic as CR excretion is not consistent and numerous studies have found considerable inter- and intra-subject variability in CR values and dependence on sex, age, activity and diet (Kesteloot and Joossens 1996). Additionally, SG was used in this study because exposure to naphthalene is known to increase creatinine levels (USEPA 1994). Urinary SG was
measured by a hand refractometer (ATAGO, PAL-095, Tokyo, Japan). Obtained mean and ranges of SG in human urine at Atonsu (1.019; [1.003-1.045]), Manhyia (1.020; [1.002-1.039]), Tafo (1.003; [1.028-1.044]) and KNUST (1.02; [1.011-1.029]) were used to adjust urinary OH-PAHs concentrations as illustrated by Nermell et al (2008). The correction formula applied to each urine concentration was as follows:

\[ \text{SG}_{\text{corrected concentration}} = \frac{(\text{SG}_{\text{target}} - 1.0)}{(\text{SG}_{\text{sample}} - 1.0)} \times \text{urinary OH-PAH concentration} \]

Where, SG\text{target} is the mean specific gravity of human urine per community; SG\text{sample} is the specific gravity of a particular sample.

2.4. Quality control and quality assurance

2.4.1. OH-PAHs

A mixture of three 13C-isotopically labelled OH-PAHs (13C6-2-OHFlu, 3-OHPhe-d9, and 13C6-1-OHPyr) was spiked into urine samples as internal standard prior to sample preparation and extraction. 13C6-2-OHFlu was used for quantification of metabolite of naphthalene and three metabolites of fluorene. 3-OHPhe-d9 was used for quantification of the five metabolites of phenanthrene and 13C6-1-OHPyr was used for quantification of 1-OHPyr, 6-OHChry, 3-OHBeP and 9-OHBaP.

Quantitation was performed using internal standard method (five-point calibration; 1, 5, 10, 50 and 100 ng/mL), and average correlation coefficients for the calibration curves in human urine were greater or equal to 0.99. The standard solutions (spiked with internal standards) for the calibration curves were prepared in urine in order to normalize differences in interferences between standards and samples. Concentrations of OH-PAHs in
urine sample used for this purpose were below the limits of detection (LOD) and differences between this and sample concentration was used in this study. Analytical methods were checked for precision and accuracy. Limits of quantification (LOQs) were calculated based on 10SD/S (SD is the standard deviation of the response of seven replicates of the lowest concentrations of each standard solution measurements and S is the slope of the calibration curve). LOQs (ng/mL) of OH-PAHs were, 0.21 (2-OHNap), 0.18 (2-3-OHFlu), 0.53 (9-OHFlu), 0.20 (2-OHPhe), 0.53 (1-9-OHPhe), 1.02 (3-OHPhe), 0.10 (4-OHPhe), 0.58 (1-OHPyr), 0.56 (6-OHChry), 0.44 (3-OHBeP) and 0.27 (9-OHBeP), respectively. Internal standard recoveries (13C6-2-OHFlu, 3-OHPhe-d9, and 13C6-1-OHPyr) ranged from 92 ± 6.5–96 ± 10.3%.

For every batch of 10 samples, a solvent blank, a spiked solvent blank (internal standards spiked into solvent), a matrix spike (internal standards spiked into blank urine), and duplicate sample were analyzed. The average recoveries in spiked solvents blanks ranged from 93 ± 5.2–98 ± 7.6%, and that for matrix spikes was 90 ± 8.9–97 ± 6.4%. Blanks were run periodically and contained no detectable amount of target analyte. The coefficients of variation (CV) of OH-PAH in duplicate samples were less than 15%.

2.4.2. MDA and 8-OHdG

Similarly, for 8-OHdG, (15N5) 8-OHdG was used as internal standard and spiked into urine samples prior to sample preparation and extraction. Quantification was performed using internal standard method (five-point calibration; 1, 5, 10, 50 and 100 ng/mL). The average correlation coefficients for the calibration curves were greater than 0.99 for both MDA and 8-OHdG. The LOQ for 8-OHdG was 0.6 ng/mL and average recovery ([15N5]
8-OHdG) was 86 ± 9.8%. For every batch of 10 samples, spiked solvent blanks (8-OHdG only) and duplicate samples were analysed and average internal standard recovery for spiked solvents blanks was 104 ± 8.7%. The CV’s for duplicate samples were less than or equal to 10% (MDA and 8-OHdG). LOQ for MDA was 0.63 μM.

1.5. Data analysis

Data analysis was performed using IBM SPSS v 20 (SPSS Inc., Illinois, USA). Kolmogorov–Smirnov (K–S) and Shapiro-Wilks tests were used to determine the normality of data and were considered statistically significant if $p$ value was less than 0.05. Data was normalized by a base 10 logarithm transformation. Concentrations of OH-PAH below their respective LOQs were replaced with a value of LOQ/2. Geometric mean concentrations (± geometric standard deviation) were used to represent the central tendency of OH-PAH in this study (Wayne 1990). Kruskal-Wallis and Wilcoxon tests were used to compare concentrations of OH-PAH in human urine from the study areas and differences were considered statistically significant with $p$ value $< 0.05$. Pearson’s correlation of logged data was used to determine the association between OH-PAHs, MDA, 8-OHdG and age. Mann-Whitney U test was also used to compare distribution of OH-PAHs, MDA and 8-OHdG between male and female participants, and considered statistically significant if $p$ value was less than 0.05. To examine the association between naphthalene exposure and occurrence of respiratory symptoms, persistent cough and headache, odds ratios (ORs) were obtained from the logistic regression models at 95% confidence interval (CI). Prior to OR calculation,
receiver operating characteristic (ROC) was used to set the threshold values for 2-OHNap which could give most accurate discrimination between the positive and negative symptoms based on the questionnaire administered and the responses in a measurement of concentration of 2-OHNap. Logistic regression analysis was performed to acknowledge the association between high exposure to 2-OHNap (over the threshold) and the number of clinical or respiratory symptoms. Statistical significance level of the tests was set at $p < 0.05$ and statistical analyses were performed JMP 10 statistical software (SAS Institute).

3. Results and discussion

3.1. Urinary levels of OH-PAHs

As shown in Table 1, 2-OHNap, 2-3-OHFlu, 1-9-OHPhe, 2-OHPhe, 4-OHPhe and 1-OHPyr were detected in human urine collected in Kumasi. Urinary 9-OHFlu and the high molecular weight, HMW ($\geq$ 4 rings) PAHs (6-OHChry, 3-OHB(e)P and 9-OHB(a)P) were not detected. This trend could be due to low detection sensitivity and/or because the HMW PAHs, such as BaP, are mainly excreted through feces (Campo et al. 2008; Li et al. 2008).

K-S and S-W’s tests for normality showed a significant variation ($p < 0.01$) in all urinary OH-PAHs measured. The detection frequencies (%) of urinary OH-PAHs and oxidative stress biomarkers in this study were 98 (2-OHNap), 87 (2-3-OHFlu), 77 (2-OHPhe), 43 (1-9-OHPhe), 2 (3-OHPhe), 80 (4-OHPhe), 39 (1-OHPyr), 95 (MDA) and 59 (8-OHdG). 3-OHPhe was not included in data and discussion because of the low detection frequency. The results of specific gravity adjusted geometric mean concentrations ($\text{GM}_{\text{SG}} \pm \text{GSD}$) showed that 2-OHNap (6.01 ± 4.21 ng/mL) was the most abundant urinary OH-PAH in humans from all study sites followed by 2-3-OHFlu (0.526 ± 4.16 ng/mL) > 2-OHPhe
(0.171 ± 3.99 ng/mL) ≥ 1-9-OHPhe (0.160 ± 3.70 ng/mL) > 4-OHPhe (0.140 ± 4.79 ng/mL) and ≥ 1-OHPyr (0.139 ± 4.44 ng/mL). The GM$_{SG}$ (± GSD) concentration (ng/mL) of the sum of OH-PAHs, $\sum$OHPAHs (2-OHNap, 2-3-OHFlu, 1-9-OHPhe, 2-OHPhe, 4-OHPhe and 1-OHPyr) were significantly higher ($p < 0.05$) in participants from urban sites compared to those from the control/reference site and decreased in the order, Manhyia (11.6 ± 3.50) > Atonsu (8.09 ± 3.35) ≥ Tafo (8.05 ± 3.24) and > KNUST (1.94 ± 1.60) (Table 1). Participants who lived within high-traffic areas could be easily affected by traffic-related airborne PAHs (Fan et al. 2012a).

3.1.1. Urinary 2-OHNaphthalene concentrations

The GM$_{SG}$ (± GSD) of urinary 2-OHNap in participants from Manhyia (8.53 ± 3.97 ng/mL) > Tafo (5.95 ± 4.06 ng/mL) ≥ Atonsu (5.62 ± 4.24 ng/mL) were significantly higher ($p < 0.05$) compared to levels detected from KNUST participants (1.37 ± 1.88) (Table 1). Highest level of 2-OHNap, (224 ng/mL), was found in urine of a 26 year old female participant in Manhyia, who reported symptoms of persistent cough, headache, tachycardia, nasal congestion and inflammation, all of which are symptoms of naphthalene exposure (USEPA 1994; Kuffner 2002). In a study by Bortey-Sam et al. (2016) urinary 2-OHNap was the most abundant OH-PAHs in cattle in Kumasi and Offinso, Ghana, and levels were attributed to vehicular emissions and use of naphthalene-containing-mothballs. The significantly higher ($p < 0.05$) levels in participants from urban sites could be due to high exposure through ingestion and/or inhalation, although 2-OHNap has been proposed as a biomarker of inhalation (Kim et al. 2000). Naphthalene is ubiquitous in ambient air, contained in cigarette smoke with high volumes in vehicular traffic (ATSDR 2005) and is
elevated in indoor air when mothballs or stoves burning biomass fuels or wood are used (Riojas-Rodriguez et al. 2011). Urinary levels of 2-OHNap are markers of vehicular traffic (Li et al. 2010) and mothball exposure (Owa et al. 1993) and correlate significantly with naphthalene vapour levels in personal air monitors (Li et al. 2010). Exposure could also be through consumption of water “purified” with naphthalene-containing-mothballs. Naphthalene is most likely the primary ingredient of mothballs in Ghana (Soghoian et al. 2012) and is frequently used in water purification (Soghoian et al. 2012), and insect repellent both in and outdoors (Bortey-Sam et al. 2016). In a study by Soghoian et al. (2012), 24% of people interviewed in a survey reported using ‘camphor’ (mothballs) routinely to purify water for drinking and bathing. Several people also gave specific recipes for making this ‘camphor water’ (mothballs), which they described as having a pleasing smell as well as internal cleansing abilities when used as a wash, gargle, drink or as an enema. Additionally, two physicians reported interviewing patients with haemolytic anaemia after ingestion of one or more whole ‘camphor’ mothballs to self-treat stomach ache, measles and diarrhoea. Exposure through ambient air is also possible because of its volatile nature as it is frequently used as freshener in clothes.

Indoor exposure to naphthalene affects more than half of the world’s population and represents a potentially important environmental contributor to the global burden of disease (Jia and Batterman 2010; Riojas-Rodriguez et al. 2011). Of the 190 urine samples collected from urban sites, 51% were above 5.8 ng/mL (SG adjusted), a value associated with chromosomal aberrations including translocation in children exposed to naphthalene, and are pre-cancerous changes in adults (Orjuela et al. 2012).
3.1.2. Urinary 1-OHPyrene concentrations

1-OHPyr is the most commonly used OH-PAH biomarker and has been used to compare exposure to PAHs between occupational and non-occupational populations (Srogi 2007). The GMSG (± GSD) of urinary 1-OHPyr detected in urban site’s participants were 0.208 ± 4.16 ng/mL (Manhyia), 0.145 ± 4.65 ng/mL (Atonsu) and 0.120 ± 4.01 ng/mL (Tafo) with no statistical difference among sites (Table 1). Concentrations of 1-OHPyr detected in urine of participants in urban sites were significantly higher ($p < 0.05$) than levels detected from KNUST participants (0.039 ± 4.17). The highest urinary concentration of 1-OHPyr (4.54 ng/mL) was from a 22 year-old participant who lived in Manhyia. The higher levels in participants from urban sites could be due to high exposure through vehicular activities or traffic. At high temperature combustion (that is during vehicular emissions) the HMW PAH compounds are dominant (Laflamme and Hites 1978). In previous studies pyrene was the eight and second most abundant PAH in PM10 and soils, respectively, in Kumasi and combustion of fuel was the dominant source (Bortey-Sam et al. 2013, 2014, 2015). Additionally, pyrene was the second most abundant in livers of wild rats in Kumasi (Bortey-Sam et al. 2015b).

3.1.3. Urinary OHPhenanthrenes and OHFluorenes concentrations

The distribution of 2-OHPhe and 4-OHPhe were not significantly different ($p > 0.05$) among participants in urban sites (Table 1). However, 1-9-OHPhe was higher ($p < 0.05$) in Manhyia participants than those in Tafo. Although not significant ($p > 0.05$), urinary concentrations of 2-OHPhe and 4-OHPhe were higher in participants from urban sites than those from KNUST. However, as shown in Table 1, 1-9-OHPhe was significantly lower ($p$
< 0.05) from KNUST participants. 1-9 and 4-OH-Phes were most abundant (4.04 and 7.19 ng/mL, respectively) in urine of a 22 year old participant who lived in Manhyia and complained of dyspnea. **Urinary concentrations of 2-3-OHFlu in participants from urban sites were significantly higher (p < 0.05) than KNUST participants.** Also, the urinary levels of 2-3-OHFlu in Manhyia participants was significantly higher (p < 0.05) than levels found in participants from the other urban sites (Table 1). In terms of abundance, the GM concentrations of OHPhe isomers did not show any significant difference (p > 0.05) although the order was 2-OHPhe (0.171 ng/mL) ≥ 1-9-OHPhe (0.160 ng/mL) ≥ 4-OHPhe (0.140 ng/mL). In a study by Fan et al. (2012a), the most dominant OHPhe isomer was 1-9-OHPhe. Similarly, Thai et al. (2016) and Levine et al. (2015) found 1-OHPhe as most dominant while Guo et al. (2013) also reported 3-OHPhe as most dominant of four phenanthrene metabolites. These variations could be due to differences in metabolism or levels of exposure (Bortey-Sam et al. 2016).

The possible source of phenanthrene and fluorene exposure in Kumasi residents could be due to inhalation during combustion at low temperatures such as wood or grass combustion since the low molecular weight, LMW (< 4 rings) PAH compounds are abundant during low temperatures combustion (Lake et al. 1979). Residents in Ghana frequently practiced bush burning including wood/grass combustion, and exposure to phenanthrenes and fluorenes through these were possible. In PM10 and soils in Kumasi, wood/grass combustion was the second dominant source of PAHs after fuel combustion (Bortey-Sam et al. 2013, 2014, 2015). Additionally, in the livers of wild rats in Kumasi,
phenanthrene was the most abundant and was significantly higher ($p < 0.05$) than other PAHs measured (Bortey-Sam et al. 2015b).

3.2. Association between urinary OH-PAHs, MDA, 8-OHdG with age

Gender and age differences have been used in various studies to predict differences in OH-PAHs concentrations in humans (Bartolomé et al. 2015; Levine et al. 2015, Sul et al. 2012). As shown in Table 2, there was no association between urinary OH-PAHs concentrations and age, which is similar to study by Thai et al. (2016). The non-linear relationship could suggest adult-specific behaviours or PAH exposure sources that are not experienced by the other age groups (ABS 2013). For example, in Ghana (WHO 2006) like Australia (ABS 2013), smoking rates were higher in adolescents (13-15%) and adults ($\geq$ 15%) than among the other age groups. Because smoking or second hand smoke is a major source of PAH exposure (Srogi 2007), different smoking rates by different age groups could have contributed to OH-PAH concentrations seen in this study (Thai et al. 2016). Of the 141 participants who answered to smoking or not in this study, 13 smoked while 128 including children did not. Additionally, according to NHANES 1999–2002 data, income level and to lesser extent education attainment, were significant determinants of urinary excretion of PAH metabolites (Suwan-ampai et al. 2009).

Among the various age groups (Table 3), there was no significant difference in all measured urinary concentrations of OH-PAHs, MDA and 8-OHdG except for 1-9-OHPhe which was significantly higher ($p < 0.05$) in age group 61-85 compared to 41-60. Although not significant, $\Sigma$OHPAHs were higher in age groups 21-40, 41-60, and 61-85 than 3-20 group with MDA and 8-OHdG highest in age groups 21-60 (Table 3). The lower
concentrations at this age (3-20 years) could reflect lower exposure to PAHs and thus potentially reduce the health effects among the group as suggested by Perera et al. (2014). This result is consistent with the 2012 Cancer Summary data from the Kumasi Cancer Registry (KsCR) (Population-based cancer registry, PBCR), where the incidence rate of cancer in Kumasi is stable or low from 0-19 years, and gradually increases with age and the peak incidence being at ages 40-64 (males) and 30-69 (females), then decreases around age 70 (KsCR (Population-based cancer registry 2012; Laryea et al. 2014). Similarly, in a study by Thai et al. (2016), urinary concentrations of OH-PAHs were low in infants (0-4), children (5-14) and the elderly (over 60 years) than in adolescents and adults (15-59 years).

3.3. Association between urinary OH-PAHs, MDA, 8-OHdG with sex

For sex differences, significantly higher ($p < 0.05$) urinary concentrations of OH-PAHs (2-OHNap, 2-3-OHFlu, 1-9-OHPhe, 4-OHPhe, 1-OHPyr, $\sum$OHPhe and $\sum$OHPAHs) were detected in females compared to males (Table 4). Although gender variation for 8-OHdG agreed with the fact that micronuclei rate (an indicator of genetic damage) was higher for females (Yang et al. 2015), urinary concentrations of MDA and 8-OHdG in this study were higher ($p > 0.05$) in male participants than females (Table 4). The difference could be impacted by many factors, including lifestyle/behaviours not captured in this study or differences in genetic factors impacting metabolism of these compounds, such as polymorphism in acetylators genes (Al-Daghri et al. 2013; Hansen et al. 2008; Levine et al. 2015). In addition to other exposure sources, this could be due to the fact that the daily tobacco smoking, which contains other contaminants other than PAHs, was 4 times higher in males (7) than females (1.7) (WHO 2011) and percent of tobacco use in persons 15 years
of age or older in Ghana was 13 times higher in males (9.5) than females (0.7) (WHO 2010). Females could also be exposed to higher levels of PAHs because they usually burned firewood/charcoal for cooking and/or cooked (fried, grilled, smoked, etc.) for their families. These activities have been associated with PAHs exposure in previous studies (Motorykin et al. 2015).

2-OH Nap was 3 times as high in females than males (Table 4). This trend is similar to studies by Orjuela et al. (2012) and Guo et al. (2014), in which the urinary levels of 1-OH Nap, 2-OH Nap, ΣOH Nap, 1-OHPhe, 9-OHPhe, ΣOHPhe, 1-OH Pyr, and ΣOH PAHs were all significantly or marginally higher among women than men workers. However, study by Thai et al. (2016) in human urine showed no association between sex and urinary OH-PAHs concentrations. When exposed to similar levels of PAHs, women had significantly higher micronuclei frequencies than men (Guo et al. 2014). Emerging evidence also indicates that women may be at greater risk of lung cancer than men, because the elevated activity of CYP1A1 enzymes in women can produce higher levels of DNA adducts, and women have lower DNA repair capacity than men (Mollerup et al. 2006; Uppstad et al. 2011). In contrast, Sul et al. (2012) and Bartolomé et al. (2015) observed significantly higher levels of urinary 2-OH Nap and ΣOHPhes, respectively, in men than females, and suggested that gender were predictors of urinary 2-OH Nap concentrations (Sul et al. 2012). These differences could be due to variations in levels and route of exposure to PAHs. Additionally, several non-pharmacogenetic factors such as age, gender, disease factors or exposure to environmental pollutants might contribute to the expression and regulation of hepatic P450 in human (Guengerich 2002; Nebbia 2001).
3.4. Association between naphthalene exposure and respiratory/clinical symptoms

For respiratory effects due to PAHs exposure, the study focused on 2-OHNap since it was the most abundant metabolite detected in human urine in Kumasi. Based on ROC, threshold values obtained were used to determine the OR (Table 5). OR analysis was used to identify the association between naphthalene exposure and occurrence of respiratory symptoms, persistent cough and headache. As shown in Table 5, exposure to naphthalene at concentrations above the threshold significantly \( p < 0.05 \) increases the odds of occurrence of tachycardia (OR = 3.36, CI: 1.39-8.1), dyspnea (OR = 3.07, CI: 1.27-7.43), persistent cough (OR = 2.68, CI: 1.43-5.05) and persistent headache (OR = 1.82, CI: 1.02-3.26) (Table 5). 2-OHNap concentration over 16.5 ng/mL increased the occurrence of asthma in both sexes though the association was not significant (OR = 5.11; CI: 0.82-31.5).

Gender differences revealed that, significant associations \( p < 0.05 \) were found between exposure to naphthalene and persistent headache (OR = 2.51, CI: 1.04-4.29) or dyspnea (OR = 3.30, CI: 1.21-8.99) in female, although not in male (Table 5). There is substantial literature showing that female animals are more susceptible to naphthalene toxicity and that repair of airway epithelium occurs more slowly than in males (Oliver et al. 2009; Van Winkle et al. 2002). Although the rates of metabolism vary during the estrous cycle, it is not clear whether gender differences in the rates of metabolism in airways entirely accounts for the higher susceptibility of females (Stelck et al. 2005). However, occurrence of persistent cough in males were more associated with urinary 2-OHNap concentrations above 8.39 ng/mL (OR = 4.44, CI: 1.05-18.6) compared with females (OR = 2.36, CI: 1.11-5.04).
Living in high-traffic areas and/or exposure to diesel exhaust particles have been associated with increased respiratory symptoms and impaired lung function in humans (Gehring et al. 2002; Wyler et al. 2000). The high occurrences and odds of respiratory symptoms, persistent cough and headache in Kumasi residents could be due to high exposure to PAHs from fuel, wood/grass combustion and also exposure through the use of naphthalene-containing-mothballs in drinking water purification, treatment of various ailment, insect repellent and/or freshener in clothes.

3.5. Human health risk implications

The study revealed that there was no significant association ($p > 0.05$) between urinary concentrations of OH-PAHs with MDA and 8-OHdG from KNUST participants (Table 2). However, at the urban sites, urinary concentrations of 2-OHNap, 2-3-OHFlu, 2-OHPhe, 1,9-OHPhe, 4-OHPhe, $\Sigma$OHpHe and $\Sigma$OHPAHs increased significantly ($p < 0.05$) as MDA increased (Table 2). Additionally, at the urban sites, there was no significant association between 8-OHdG and the OH-PAHs studied except 4-OHPhe ($p < 0.05$). This trend is quite similar to previous studies where urinary 8-OHdG and/or MDA gradually increased as the environmental exposure levels to PAHs increased (Kuang et al. 2013; Li et al. 2015; Yang et al. 2015) although strong correlation between urinary $\Sigma$OHPAHs and 8-OHdG was not established in non-occupational population (Fan et al. 2012b). MDA has potential to react with nucleic acid bases to form DNA adducts, create DNA interstand crosslinks, and even generate DNA protein cross-links (Del Rio et al. 2005). These reaction products have been demonstrated to be involved in the onset and development of a series of adverse health
effects including cardiovascular diseases, diabetes and cancers (Dierckx et al. 2003; Wu et al. 2004).

Similarly, urinary 8-OHdG have also been indicated as a risk factor for atherosclerosis, diabetes, and various cancers (Wu et al. 2004). Increased oxidative stress levels reportedly correlated with many chronic degenerative diseases such as allergic inflammatory diseases (Bartsch and Nair 2004), obesity and atherosclerosis (Kobayashi et al. 2011; Wu et al. 2004) which were some of the symptoms obtained from participants in this study during the face-to-face interview. For instance, the questionnaire revealed that 10% of participants were diabetics and 2% had arthritis. Of 33 participants that we calculated body mass index, 33% (11) were overweight and 21% (7) were obese. In previous studies, a positive association was observed between urinary biomarkers of 1 and 2-hydroxynapthol, 2-OHPhe and summed low molecular weight (LMW) PAH biomarkers, and diabetes mellitus (Alshaarawy et al. 2014). Additionally, a positive relationship between exposure to PAHs and obesity in American children and adolescents was observed with PAHs acting as obesogens (Irigaray et al. 2006; Kim et al. 2014; Scinicariello et al. 2014). Further, the high prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in the Ghanaian population could increase the risk of toxic effects from ingestion of mothballs or other naphthalene-containing products (Soghoian et al. 2012).

4. Conclusions

Urinary 2-OHNap was the most abundant OH-PAH excreted in humans in Kumasi, Ghana and naphthalene exposure through vehicular emissions and the frequent use of naphthalene-containing-mothballs in drinking water purification, insect repellent, freshener
in clothes have contributed significantly. **Urinary concentrations of the ∑OH-PAHs were significantly higher from urban sites compared to the control site.** The present study showed a significant sex difference with higher levels of urinary OH-PAHs in women than men. The study revealed that exposure to naphthalene significantly increases the occurrence of persistent cough, persistent headache, tachycardia and dyspnea. Gender differences revealed that the occurrence of persistent headache and dyspnea due to naphthalene exposure were significantly higher in females than males. There was no association between urinary OH-PAHs concentrations and age, with MDA and 8-OHdG highest in age groups 21-60. The high prevalence of G6PD deficiency in the Ghanaian population and the significantly positive association between urinary MDA, 8-OHdG and OH-PAHs concentrations could suggest lipid peroxidation/cell damage or chronic degenerative diseases including cardiovascular diseases to Kumasi residents through PAHs exposure.
Acknowledgements

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References


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Figure captions:

Fig. 1 Map showing human urine sampling locations in Kumasi, Ghana (yellow pins indicate city centre and environs/sites contaminated with PAHs; red pin indicate human urine sites; white pin indicate KNUST campus)
Table 1: Specific gravity adjusted OH-PAHs concentrations (ng/mL) in human urine in Kumasi, Ghana

<table>
<thead>
<tr>
<th>Sample sites</th>
<th>n</th>
<th>2-OHNap</th>
<th>2-3-OHFlu</th>
<th>2-OHPhe</th>
<th>1-9-OHPhe</th>
<th>4-OHPhe</th>
<th>1-OHPyr</th>
<th>ΣOHPhe</th>
<th>ΣOHPAHs</th>
<th>MDA</th>
<th>8-0HdG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atonsu</strong></td>
<td>82</td>
<td>GM</td>
<td>5.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.521&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.181&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.172&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.122&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.958&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>0.231&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.204&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.601</td>
<td>4.20</td>
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n: number of samples; nd: below limits of quantification (LOQ); GM: geometric mean concentration adjusted by specific gravity; GSD: geometric standard deviation; # indicates concentrations in micro molar (µM); different letters (a, b and c) within a column indicates significant differences (p < 0.05)
Table 2: Correlation between urinary concentrations (ng/mL) of OH-PAHs, MDA, 8-OHdG and age of participants in Kumasi, Ghana

<table>
<thead>
<tr>
<th></th>
<th>2- OHNap</th>
<th>2-3- OHFlu</th>
<th>2- OHPhe</th>
<th>1-9- OHPhe</th>
<th>4- OHPhe</th>
<th>1- OHPyr</th>
<th>ΣOHPhe</th>
<th>ΣOHPAHs</th>
<th>MDA</th>
<th>8- OHdG</th>
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<td>0.229**</td>
<td>0.239**</td>
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<td>4-OHPhe</td>
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<td>0.726**</td>
<td>0.879**</td>
<td>0.883**</td>
<td>0.854**</td>
<td>0.767**</td>
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<tr>
<td>ΣOHPAHs</td>
<td>0.966**</td>
<td>0.674**</td>
<td>0.604**</td>
<td>0.552**</td>
<td>0.545**</td>
<td>0.546**</td>
<td>0.639**</td>
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</tr>
<tr>
<td>MDA</td>
<td>0.359**</td>
<td>0.223**</td>
<td>0.399**</td>
<td>0.200**</td>
<td>0.146*</td>
<td>0.136</td>
<td>0.216**</td>
<td>0.362**</td>
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<tr>
<td>8-OHdG</td>
<td>0.12</td>
<td>0.119</td>
<td>0.074</td>
<td>0.062</td>
<td>0.149*</td>
<td>0.088</td>
<td>0.123</td>
<td>0.138</td>
<td>0.088</td>
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<tr>
<td>Age (yrs)</td>
<td>0.159</td>
<td>0.158</td>
<td>0.163</td>
<td>0.087</td>
<td>0.074</td>
<td>0.051</td>
<td>0.132</td>
<td>0.189</td>
<td>0.009</td>
<td>0.138</td>
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</tr>
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<thead>
<tr>
<th></th>
<th>2- OHNap</th>
<th>2-3- OHFlu</th>
<th>2- OHPhe</th>
<th>1-9- OHPhe</th>
<th>4- OHPhe</th>
<th>1- OHPyr</th>
<th>ΣOHPhe</th>
<th>ΣOHPAHs</th>
<th>MDA</th>
<th>8- OHdG</th>
<th>Age (yrs)</th>
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<tbody>
<tr>
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<tr>
<td>2-OHPhe</td>
<td>0.402</td>
<td>0.678</td>
<td>1</td>
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<tr>
<td>1-9-OHPhe</td>
<td>0.774</td>
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<td>0.270</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4-OHPhe</td>
<td>0.826</td>
<td>0.630</td>
<td>0.0637</td>
<td>0.532</td>
<td>1</td>
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<td></td>
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<tr>
<td>1-OHPyr</td>
<td>0.936*</td>
<td>0.956*</td>
<td>0.668</td>
<td>0.804</td>
<td>0.647</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>ΣOHPhe</td>
<td>0.835</td>
<td>0.956*</td>
<td>0.813</td>
<td>0.733</td>
<td>0.506</td>
<td>0.974**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΣOHPAHs</td>
<td>0.999**</td>
<td>0.848</td>
<td>0.418</td>
<td>0.761</td>
<td>0.821</td>
<td>0.938*</td>
<td>0.8414</td>
<td>1</td>
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</tr>
<tr>
<td>MDA</td>
<td>-0.0865</td>
<td>-0.0149</td>
<td>0.537</td>
<td>-0.557</td>
<td>-0.0016</td>
<td>0.0069</td>
<td>0.1275</td>
<td>-0.0635</td>
<td>1</td>
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</tr>
<tr>
<td>8-OHdG</td>
<td>-0.0937</td>
<td>0.149</td>
<td>0.0956</td>
<td>-0.151</td>
<td>0.331</td>
<td>-0.0674</td>
<td>-0.0175</td>
<td>-0.0967</td>
<td>0.434</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Age/years</td>
<td>-0.4345</td>
<td>-0.5213</td>
<td>-0.5474</td>
<td>-0.0315</td>
<td>-0.6180</td>
<td>-0.4627</td>
<td>-0.4995</td>
<td>-0.4449</td>
<td>-0.719</td>
<td>-0.718</td>
<td></td>
</tr>
</tbody>
</table>

* Indicates significance at $p < 0.05$; ** Indicates significance at $p < 0.01$
Table 3: Age differences in urinary concentrations (ng/mL) of OH-PAHs, MDA, and 8-OHdG among participants in Kumasi, Ghana

<table>
<thead>
<tr>
<th>Age groups</th>
<th>n</th>
<th>2-OHNap</th>
<th>2-3-OHFlu</th>
<th>2-OHPhe</th>
<th>1-9-OHPhe</th>
<th>4-OHPhe</th>
<th>1-OHPyr</th>
<th>ΣOHPhe</th>
<th>ΣOHPAHs</th>
<th>MDA#</th>
<th>8-OHdG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages 3-20</td>
<td>GM</td>
<td>36</td>
<td>4.99(^a)</td>
<td>0.501(^a)</td>
<td>0.145(^a)</td>
<td>0.134(^{ab})</td>
<td>0.113(^a)</td>
<td>0.141(^a)</td>
<td>0.576(^a)</td>
<td>6.94(^a)</td>
<td>0.813(^a)</td>
</tr>
<tr>
<td></td>
<td>GSD</td>
<td>3.84</td>
<td>3.75</td>
<td>3.81</td>
<td>3.93</td>
<td>3.38</td>
<td>3.31</td>
<td>2.61</td>
<td>3.21</td>
<td>4.23</td>
<td>1.99</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>0.709</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.137</td>
<td>1.16</td>
<td>nd</td>
<td>0.206</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>62.6</td>
<td>3.46</td>
<td>1.08</td>
<td>1.42</td>
<td>0.922</td>
<td>0.922</td>
<td>3.46</td>
<td>63.1</td>
<td>8.38</td>
<td>2.01</td>
</tr>
<tr>
<td>Ages 21-40</td>
<td>GM</td>
<td>94</td>
<td>5.28(^a)</td>
<td>0.552(^a)</td>
<td>0.170(^a)</td>
<td>0.174(^{ab})</td>
<td>0.147(^a)</td>
<td>0.143(^a)</td>
<td>0.716(^a)</td>
<td>7.69(^a)</td>
<td>0.914(^a)</td>
</tr>
<tr>
<td></td>
<td>GSD</td>
<td>4.04</td>
<td>4.61</td>
<td>4.16</td>
<td>3.93</td>
<td>5.72</td>
<td>5.53</td>
<td>2.88</td>
<td>3.33</td>
<td>2.01</td>
<td>2.52</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.102</td>
<td>0.511</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>224</td>
<td>8.88</td>
<td>2.81</td>
<td>4.04</td>
<td>7.19</td>
<td>4.54</td>
<td>14.2</td>
<td>230</td>
<td>7.56</td>
<td>12.9</td>
</tr>
<tr>
<td>Ages 41-60</td>
<td>GM</td>
<td>29</td>
<td>9.88(^a)</td>
<td>0.652(^a)</td>
<td>0.251(^a)</td>
<td>0.125(^b)</td>
<td>0.114(^a)</td>
<td>0.137(^a)</td>
<td>0.654(^a)</td>
<td>12.8(^a)</td>
<td>0.928(^a)</td>
</tr>
<tr>
<td></td>
<td>GSD</td>
<td>4.97</td>
<td>2.83</td>
<td>3.04</td>
<td>3.73</td>
<td>5.30</td>
<td>3.23</td>
<td>2.31</td>
<td>3.50</td>
<td>1.97</td>
<td>2.93</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.137</td>
<td>0.644</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
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<td>2.85</td>
<td>2.84</td>
<td>0.575</td>
<td>0.772</td>
<td>0.622</td>
<td>5.68</td>
<td>155</td>
<td>6.59</td>
<td>15.6</td>
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<tr>
<td>Ages 61-85</td>
<td>GM</td>
<td>31</td>
<td>9.10(^a)</td>
<td>0.745(^a)</td>
<td>0.240(^a)</td>
<td>0.369(^a)</td>
<td>0.216(^a)</td>
<td>0.224(^a)</td>
<td>1.03(^a)</td>
<td>12.7(^a)</td>
<td>0.751(^a)</td>
</tr>
<tr>
<td></td>
<td>GSD</td>
<td>4.96</td>
<td>4.56</td>
<td>4.18</td>
<td>3.16</td>
<td>5.73</td>
<td>4.40</td>
<td>3.07</td>
<td>4.20</td>
<td>1.58</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.182</td>
<td>0.549</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td></td>
<td>Maximum</td>
<td>117</td>
<td>6.54</td>
<td>1.67</td>
<td>2.02</td>
<td>2.49</td>
<td>2.49</td>
<td>5.24</td>
<td>125</td>
<td>1.45</td>
<td>1.81</td>
</tr>
</tbody>
</table>

n: number of samples; nd: below limit of quantification; GM: geometric mean concentration adjusted by specific gravity; GSD: geometric standard deviation; \# indicates concentrations in micro molar (μM); different letters (a and b) within a column indicates significant differences (p < 0.05) among age groups.
Table 4: Distribution of SG adjusted urinary OHPAHs, MDA and 8-OHdG concentrations (ng/mL) in males and females in Kumasi, Ghana

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>n</th>
<th>2-OHNap</th>
<th>2-3-OHFlu</th>
<th>2-OHPhe</th>
<th>1-9-OHPhe</th>
<th>4-OHPhe</th>
<th>1-OHPyr</th>
<th>ΣOHPhe</th>
<th>ΣOHPAHs</th>
<th>MDA</th>
<th>8-OHdG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>3-20</td>
<td>12</td>
<td>3.81</td>
<td>0.344</td>
<td>0.148</td>
<td>0.135</td>
<td>0.0967</td>
<td>0.129</td>
<td>0.521</td>
<td>5.29</td>
<td>8.9</td>
<td>0.993</td>
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<tr>
<td></td>
<td>21-40</td>
<td>24</td>
<td>2.62</td>
<td>0.340</td>
<td>0.108</td>
<td>0.0801</td>
<td>0.075</td>
<td>0.0557</td>
<td>0.418</td>
<td>3.92</td>
<td>1.07</td>
<td>3.16</td>
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<td></td>
<td>41-60</td>
<td>9</td>
<td>3.41</td>
<td>0.547</td>
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<td>0.127</td>
<td>0.0836</td>
<td>0.148</td>
<td>0.672</td>
<td>5.77</td>
<td>0.85</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>61-85</td>
<td>12</td>
<td>3.18</td>
<td>0.202</td>
<td>0.209</td>
<td>0.179</td>
<td>0.187</td>
<td>0.134</td>
<td>0.730</td>
<td>4.83</td>
<td>0.76</td>
<td>1.01</td>
</tr>
<tr>
<td>Females</td>
<td>3-20</td>
<td>12</td>
<td>8.27</td>
<td>0.666</td>
<td>0.188</td>
<td>0.188</td>
<td>0.165</td>
<td>0.169</td>
<td>0.774</td>
<td>11.1</td>
<td>2.08</td>
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<tr>
<td></td>
<td>21-40</td>
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<td>6.89</td>
<td>0.66</td>
<td>0.202</td>
<td>0.234</td>
<td>0.190</td>
<td>0.203</td>
<td>0.879</td>
<td>9.94</td>
<td>0.86</td>
<td>1.56</td>
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<td></td>
<td>41-60</td>
<td>20</td>
<td>16.3</td>
<td>0.705</td>
<td>0.226</td>
<td>0.125</td>
<td>0.133</td>
<td>0.133</td>
<td>0.646</td>
<td>18.6</td>
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<td>3.17</td>
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<td>0.142</td>
<td>0.169</td>
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<td>0.139</td>
<td>0.679</td>
<td>10.2</td>
<td>0.66</td>
<td>0.952</td>
</tr>
</tbody>
</table>

n: number of samples; nd: below limit of quantification; GM: geometric mean concentration adjusted by specific gravity; GSD: geometric standard deviation; different letters (a and b) within a column indicate significant differences (Student’s T-Test; p < 0.05); # indicates concentrations in micro molar (uM)
Table 5: OR (95% CI) for the presence or absence of clinical/respiratory symptoms in Kumasi residents due to naphthalene exposure

<table>
<thead>
<tr>
<th>OHPAH</th>
<th>Clinical symptom</th>
<th>Both sexes</th>
<th>Male</th>
<th>Female</th>
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<tr>
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<td>Threshold</td>
<td>OR</td>
<td>CI</td>
<td>p value</td>
</tr>
<tr>
<td>2-OHNP</td>
<td>Persistent cough</td>
<td>8.39</td>
<td>2.68</td>
<td>1.43-5.05</td>
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<td></td>
<td>Persistent headache</td>
<td>7.55</td>
<td>1.82</td>
<td>1.02-3.26</td>
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<tr>
<td></td>
<td>Asthma</td>
<td>16.5</td>
<td>5.11</td>
<td>0.82-31.5</td>
</tr>
<tr>
<td></td>
<td>Wheezing</td>
<td>7.08</td>
<td>1.18</td>
<td>0.07-19.2</td>
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<tr>
<td>Respiratory tract infection</td>
<td>3.11</td>
<td>0.31</td>
<td>0.03-2.71</td>
<td>0.26</td>
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<tr>
<td>Upper respiratory congestion</td>
<td>8.88</td>
<td>3.36</td>
<td>1.39-8.1</td>
<td>0.004</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>6.6</td>
<td>3.07</td>
<td>1.27-7.43</td>
<td>0.0098</td>
</tr>
</tbody>
</table>

OR: Odds ratio; CI: 95% Confidence Interval; Threshold values were expressed in ng/mL; NCI: nasal congestion or inflammation
Fig. 1