Excretion of polycyclic aromatic hydrocarbon metabolites (OH-PAHs) in cattle urine in Ghana

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Abstract

Previous studies of polycyclic aromatic hydrocarbons (PAHs) in particulate matter, soils and livers of wild rats indicated that the city centre of Kumasi, Ghana has been severely polluted with high cancer potency. Cattle urine were therefore collected from Kumasi (urban) and Offinso (rural), Ghana: to determine concentrations of urinary PAH metabolites (OH-PAHs); and find their association with sex; and to estimate exposure of cattle to PAHs from the different sites. From the results, geometric mean concentrations (adjusted by specific gravity), GM_{SG}, showed that 2-OHNaphthalene (2-OHNap) was the most abundant OH-PAH in cattle urine from all study sites, and naphthalene-containing-mothballs might have contributed significantly to the levels. There was no significant difference between urinary OH-PAHs concentrations in cattle from urban and rural sites except for 2-OHPhe and 4-OHPhe, and similar to urban areas, rural sites could also be polluted with PAHs. GM_{SG} of 2-OHNap in cattle urine in Kokote (21.9 ± 6.51 ng/mL; a rural area), was significantly higher compared to the other sites followed by Oforikrom (4.15 ± 4.37 ng/mL; urban). The GM_{SG} concentration (ng/mL) of the sum of OH-PAHs decreased in the order, Kokote (44.7) > Oforikrom (7.87) > Saboa (6.98) > Santasi (6.68) > and Twumasen Estate (5.23). The high concentrations of urinary 2-OHNap, 2-3-OHFlu, 2-OHPhe, 3-OHPhe and 4-OHPhe in Kokote indicated high PAHs exposure to cattle in this area or different/specific source of PAHs exposure. GM_{SG} of 2-OHNap was significantly higher in male cattle compared to females while 1-9-OHPhe was significantly higher in females.

Capsule:

PAH metabolites were measured in cattle urine in urban and rural areas in Ghana; 2-OHNaphthalene (2-OHNap) was the most abundant PAH metabolites.

Keywords: OH-PAHs; Kumasi; Metabolites; Cattle; Urine

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are pollutants formed during incomplete combustion of organic materials. They are found in vehicle exhaust, wood and cigarette smoke, and also in 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; Dissanayake and Galloway, 2004). According to the Agency for Toxic Substances and Disease Registry's (ATSDR, 2013) priority list of hazardous chemicals, PAHs were classified as the 9th most hazardous chemical. In humans and animals, PAHs are metabolized by cytochrome P450 enzymes and excreted in urine. One of the major metabolites is monohydroxylated PAH (OH-PAHs) (Burczynski et al., 1998) and urinary levels of 1-hydroxypyrene has been used as biomarker of PAHs exposure (Bouchard and Viau, 1999; Jongeneelen, 2001). However, since ratios of different PAHs may vary depending on the source and personal enzymatic capacity, concentration profiles of multiple OH-PAHs biomarkers are necessary and required to assess the environmental exposure risk. Urinary metabolites of naphthalene, fluorene and phenanthrene are also commonly used as biomarkers to assess the exposure level and environmental risk (Li et al., 2008; Fan et al., 2012; 2012b).

63 The assessment of health risk to humans exposed to PAHs is primarily based on results
64 from animal studies, which indicated that PAHs can produce carcinogenic and mutagenic
65 effects. Recent studies also indicated that some PAH metabolites have strong correlation
66 with atherosclerosis and cardiovascular diseases (Xu et al., 2010), and exposure of rats and
67 mice to naphthalene caused nasal and bronchiolar tumors, respectively (NTP, 1992; 2000).
68 As a developing country, the economic and population growth rates in Ghana have seen
69 tremendous increases over the past few years. The growing rate of industrialization is
70 gradually leading to contamination and deterioration of the environment and pollution is
71 likely to reach disturbing levels (Bortey-Sam et al., 2014). Studies by Bortey-Sam et al.
72 (2013; 2014; 2015) in particulate matter (PM10) and soils indicated that the city centre of
73 Kumasi, Ghana has been polluted with PAHs when compared with recommended levels,
74 and fuel and wood/grass combustion were the dominant sources. The total benzo(a)pyrene
75 equivalent concentration and estimated carcinogenicity of PAHs in PM10 and soil from the
76 city centre was approximately 18 and 150 times higher, respectively, as compared to a
77 pristine site. Rats were therefore used as sentinels to measure the environmental pollution
78 state, and higher levels of PAHs were detected in the livers of wild rats in the city centre of
79 Kumasi. Naphthalene was detected in 80% of those samples, and levels of phenanthrene
80 and pyrene (the first and second most abundant, respectively) were significantly higher than
81 other PAHs measured (Bortey-Sam et al., 2015b).
82 Based on the high levels and cancer potency of PAHs in PM10, soils and the levels
83 found in livers of wild rats (Bortey-Sam et al. 2013; 2014; 2015; 2015b), cattle urine was
84 collected because cattle is known to excrete large amount of PAH metabolites due to high

intake of the parent compound through feed or inhalation (Saengtienchai et al., 2014). PAHs in the atmosphere are known to settle in soil (Rey-Salgueiro et al. 2008) and this could increase the levels of exposure in Kumasi, Ghana, because these free-range cattle also pick food and/or water from the ground. Urinary levels of PAHs could be widely used as biological indicator of exposure (Jongeneelen, 2001), and there is limited/no data from literature that addresses the excretory levels of OH-PAHs in cattle in Ghana. The objectives of the present study were therefore: to determine the concentrations of OH-PAHs in cattle urine in Kumasi (urban) and Offinso (rural), Ghana; find the association between urinary OH-PAHs concentrations and sex; and to estimate cattle's exposure to PAHs from the different sites.

2. Materials and methods

2.1. Sampling

In August 2014, urine samples of healthy cattle (West African Shorthorn) were randomly collected from 5 communities in Kumasi and Offinso, both in the Ashanti Region of Ghana. Offinso is about 33 km from the city centre of Kumasi (Fig. 1). Samples were collected from Oforikrom and Santasi in Kumasi (urban), which are 5.1 and 3.5 km from the city centre, respectively (Fig. 1), where previous studies reported high levels of PAHs in PM10, soils and livers of wild rats (Bortey-Sam et al., 2013; 2014; 2015; 2015b). On the other hand, the three sites in Offinso (Twumasen Estate, Saboa and Kokote) selected for cattle urine sampling (Fig. 1) are in rural and agricultural areas where bush burning is rampant and the use and sometimes abuse of pesticides such as carbaryl (1-naphthyl-N-methylcarbamate), which could be metabolized to 1-hydroxy naphthalene, was possible

(Meeker et al., 2007; Orjuela et al., 2012). In addition, due to the lack of background urine and interferences during OH-PAHs quantification, 500 mL of cattle urine (blank stock) was collected from Hokkaido University School farm. Hokkaido University is a public university located in Sapporo, Japan, and because of the low vehicular movement and industrial activities around the farm, PAHs exposure from point sources were assumed to be negligible. However, because cattle could be exposed to PAHs through feed and/or inhalation the sample collected was measured several times to confirm levels of OH-PAHs.

From the sample sites in Ghana, spot urine (n = 95; with 30 males and 65 females) were collected, transferred into labelled corning tubes (Corning Incorporated, New York, USA) and stored at –20 ^{o}C in the Department of Chemistry, Kwame Nkrumah University Science and Technology (KNUST), Ghana. Of the 5 sites, only ages of some cattle in 2 sites (Twumasen Estate and Saboa) were obtained from the herdsmen. The average ages (ranges) of cattle were 2.9 ± 1.0 years (1-4.5years) in Twumasen Estate and 4.2 ± 2.9 years (1-12 years) in Saboa, respectively. Samples were later transported to the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan where they were stored at –30 ^{o}C until analysis (quarantine number for importing is 26 douken 383).

2.2. Sample extraction and analysis

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-

129 1OHPyrene). The pH of sample was adjusted to 5.5 using 1 M acetic acid (Wako Pure
130 Chemicals, Osaka, Japan) and incubated overnight at 37 °C. The sample was diluted with 4
131 mL of Milli-Q water and extracted twice (liquid-liquid extraction) with 10 mL each of n-
132 pentane (Kanto Chemical Corp., Tokyo, Japan) by shaking for 1 h. To reduce the
133 interference of sulfur metabolites, the combined extracts were washed with 2 mL of 1 N
134 $AgNO_3$ solution (Wako Pure Chemicals, Osaka, Japan), concentrated to 50-100 µL,
135 redissolved to 0.5 mL using methanol and filtered (0.20 µm DISMIC-13JP membrane filter,
136 ADVANTEC, Toyo Roshi Kaisha Ltd., Japan) prior to instrumental analysis. All sample
137 preparation steps were performed in darkness (by covering tubes completely with
138 aluminum foil) to avoid possible photodegradation of target analytes. A total of 13 OH-
139 PAHs; 2-hydroxynaphthalene (2-OHNap), 2-hydroxyfluorene (2-OHFlu), 3-
140 hydroxyfluorene (3-OHFlu), 9-hydroxyfluorene (9-OHFlu), 1-hydroxyphenanthrene (1-
141 OHPhe), 2-hydroxyphenanthrene (2-OHPhe), 3-hydroxyphenanthrene (3-OHPhe), 4-
142 hydroxyphenanthrene (4-OHPhe), 9-hydroxyphenanthrene (9-OHPhe), 1-hydroxypyrene
143 (1-OHPyr), 6-hydroxychrysene (6-OHChry), 3-hydroxybenzo(e)pyrene (3-OHBeP) and 9-
144 hydroxybenzo(a)pyrene (9-OHBaP), were analyzed in each sample. The standards (purity ≥
145 98%) were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA) and
146 Toronto Research Chemical Inc. (Brisbane Road, North York, Canada). Difficulties were
147 often associated with the separation of 1-OHPhe and 9-OHPhe, for this reason, the sum of
148 these isomers was used as an abbreviation, 1-9-OHPhe. Similarly, 2-3-OHFlu was used as
149 sum of 2-OHFlu and 3-OHFlu. All results were adjusted by specific gravity and expressed
150 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.

2.3. Specific gravity (SG) of cattle urine

To compensate for variations in urine dilution, urinary OH-PAH concentrations were adjusted by specific gravity (SG). Urinary SG was measured by a hand refractometer (ATAGO, PAL-095, Tokyo, Japan). Obtained mean and ranges of SG in urine of cattle in Oforikrom (1.013; [1.004-1.029]), Santasi (1.035; [1.03-1.041]), Twumasen Estate (1.035; [1.028-1.04]), Saboa (1.036; [1.026-1.049]) and Kokote (1.037; [1.029-1.042]) 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:

$$\text{SG\_corrected concentration} = \text{urinary OH} - \text{PAH concentration} \times \frac{(\text{SG}_{\text{target}} - 1.0)}{(\text{SG}_{\text{sample}} - 1.0)}$$

Where, SG_{target} is the mean specific gravity of cattle urine per community; SG_{sample} is the specific gravity of a particular sample.

2.4. Quality control and quality assurance

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 1OHPyr, 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 (r^2) for the calibration curves in cattle 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 replicate standard solution measurements and S is the slope of the calibration curve). LOQs (ng/mL) of OH-PAHs were, 0.29 (2-OHNap), 0.24 (2-3-OHFlu), 0.60 (9-OHFlu), 0.23 (2-OHPhe), 0.71 (1-9-OHPhe), 1.16 (3-OHPhe), 0.15 (4-OHPhe), 0.87 (1-OHPyr), 0.73 (6-OHChry), 0.54 (3-OHBeP) and 0.32 (9-OHBeP), respectively. Internal standard recoveries (13C6-2OHFlu, 3-OHPhe-d9, and 13C6-1-OHPyr) ranged from 89 ± 5.8–96 ± 11.4% (Table 1).

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 92 ± 4.6–98 ± 8.3%, and that for matrix spikes was 85 ± 9.1–96 ± 7.1%. Blanks were run periodically and contained no detectable amount of target analyte. The coefficients of variation of OH-PAH in duplicate samples were less than 15%.

1.5. Data analysis

Data analysis was performed using IBM SPSS v 20 for windows (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. Concentrations of OH-PAH below their respective LOQs were replaced with a value of LOQ/2. Geometric mean concentrations were used to represent the central tendency of OHPAH in this study (Wayne, 1999). ANOVA and Tukey analyses of log transformed data were used to compare concentrations of OH-PAH in cattle urine from the study areas and differences were considered statistically significant with p value < 0.05. Student's T-Test was also used to compare distribution of OH-PAHs between male and female cattle; and, urban and rural sites.

3. Results and discussion

3.1. Excretory levels of OH-PAH in cattle urine

As shown in Table 2, there was no significant difference ($p > 0.05$) between urinary OH-PAHs concentrations in cattle from urban and rural sites except for 2-OHPhe and 4-OHPhe. Data for 3-OHPhe was not included because concentrations from urban sites and 2

213 rural sites were below the LOQ (Table 3). The significant differences ($p < 0.05$) could
214 indicate differences in cattle's exposure to PAHs from urban and rural sites. Table 3 shows
215 the distribution of OH-PAHs in cattle urine from the 5 sample sites. From the results, 2-
216 OHNap, 2-3-OHFlu, 1-9-OHPhe, 2-OHPhe, 3-OHPhe, 4-OHPhe and 1-OHPyr were
217 detected. 9-OHFlu and the high molecular weight, HMW (≥ 4 rings) PAHs (6-OHChry, 3-
218 OHB(e)P and 9-OHB(a)P) were however not detected in the cattle urine and could be due
219 to low detection sensitivity (Campo et al., 2008) and/or because the HMW PAHs, such as
220 BaP, are mainly excreted through feces (Burgaz et al. 1992; Li et al., 2008).

221 Specific gravity adjusted geometric mean concentrations (GM_{SG}) showed that 2-OHNap
222 (2.77 ± 5.91 ng/mL; $p < 0.01$) was the most abundant OH-PAH in cattle urine from all
223 study sites followed by 1-9-OHPhe (2.02 ± 1.16 ng/mL) > 4-OHPhe (1.74 ± 1.87 ng/mL) >
224 1-OHPyr (1.22 ± 0.87 ng/mL) > 2-3-OHFlu (1.08 ± 1.75 ng/mL) > 2-OHPhe (0.489 ±
225 0.555 ng/mL) > and 3-OHPhe (0.278 ± 0.553 ng/mL) (Fig. 2). The GM_{SG} concentration
226 (ng/mL) of the sum of OH-PAHs (2-OHNap, 2-3-OHFlu, 1-9OHPhe, 2-OHPhe, 3-OHPhe,
227 4-OHPhe and 1-OHPyr) decreased in the order, Kokote (44.7 ± 10.4) > Oforikrom (7.87 ±
228 7.41) > Saboa (6.98 ± 3.86) > Santasi (6.68 ± 2.701) > and Twumasen Estate (5.23 ± 1.55).
229 High urinary concentrations of 2-OHNap, 2-3-OHFlu, 2-OHPhe, 3-OHPhe and 4-OHPhe
230 were detected in Kokote (Table 3) indicating high exposure of cattle to the parent PAHs
231 within the sample site. Kokote is a rural area filled with many farmlands, with high
232 agricultural and burning activities compared to the other sites, and the levels of OH-PAHs
233 could mean that there were different or specific sources of PAHs exposure to cattle in the
234 area.

235 *3.2. 2-OHNaphthalene*

236 The GM_{SG} of 2-OHNap in cattle urine in Kokote (21.9 ± 6.51 ng/mL) was significantly
237 higher ($p < 0.05$) compared to the other sites followed by Oforikrom (4.15 ± 4.37 ng/mL)
238 (Table 3). However, the least GM_{SG} concentration for 2-OHNap was recorded in Santasi
239 (0.61 ± 0.23 ng/mL). Although 1-OHNap could be derived from both naphthalene and
240 carbaryl, 2-OHNap is derived only from naphthalene (Orjuela et al., 2012). The high levels
241 of 2-OHNap could be due to exposure through ingestion and/or inhalation, although it has
242 been proposed as a biomarker of inhalation (Kim et al. 2000). Naphthalene is ubiquitous in
243 ambient air with high volumes in vehicular traffic, cigarette smoke (ATSDR, 2005) and is
244 elevated when mothballs or stoves burning biomass fuels are used (Griego et al., 2008;
245 Riojas-Rodriguez et al., 2011). Urinary levels of 2-OHNap are markers of vehicular traffic
246 (Li et al., 2010) and mothball exposure (Owa et al., 1993). Naphthalene is most likely the
247 primary ingredient of mothballs in Ghana (Soghoian et al., 2012), and is frequently used in
248 driving away insects both in and outdoors. This practice could also contribute to 2-OHNap
249 being the most abundant metabolite in cattle urine since exposure through ambient air was
250 also possible because of its volatile nature.

251 *3.3. 1-OHPyrene*

252 The highest GM_{SG} concentration of 1-OHPyr were detected in cattle in Kokote (2.29 ±
253 1.28 ng/mL) and Oforikrom (1.37 ± 1.18 ng/mL). Levels in Kokote were significantly
254 higher ($p < 0.05$) compared to other sites except Oforikrom and Santasi (Table 3). GM
255 concentrations (not adjusted by SG) of 1-OHPyr in this study (1.02 ng/mL [Twumasen
256 Estate] to 2.41 ng/mL [Kokote]) were generally higher compared to study by Saengtienchai

et al. (2014) using cattle from Japan and Thailand (non-adjusted). A study by Ferrari et al. (2002) on determination of 1-OHPyr in bovine urine from three farms located near rural and urban areas recorded non-adjusted average concentrations of 0.66 ng/mL (urban area); 1.52 ng/mL (rural) and 5.09 ng/mL (highway). Similar to the present study, Ferrari et al. (2002) indicated higher levels of 1-OHPyr in rural areas compared to urban and suggested that other important sources besides traffic could contribute to the PAHs burden of animals. Results of non-adjusted 1-OHPyr concentrations from the present study was higher compared to study by Ferrari et al. (2002) except for levels recorded in cattle raised on farms located in the vicinity of a highway (5.09 ng/mL).

The levels in the present study could be due to vehicular activities or traffic. At high temperature combustion (that is during vehicular emissions) the HMW PAH compounds are dominant (Laflamme and Hites, 1978). Previous studies by Bortey-Sam et al. (2014; 2015) in PM10 and soils indicated pyrene as the eighth and second most abundant PAH in Kumasi, respectively, and combustion of fuel (74%) and wood/grass (23%) were the dominant sources in the region. In Ghana, some farms are generally located close to major roads with high vehicular activities or traffic (Tay and Biney, 2013), and exposure to domestic and grazing animals could be through inhalation, or picking food or water from the ground.

3.4. OHPhenanthrenes and OHFluorenes

The distribution of 2-OHPhe, 4-OHPhe and 2-3-OHFlu were significantly higher ($p < 0.05$) in Kokote than the other sites (Table 3). Urinary levels of 1-9-OHPhe in Kokote was however significantly higher ($p < 0.05$) than levels found in Oforikrom. 3-OHFlu in cattle

279 urine from all sites were below the LOQ except Kokote (Table 3). In this study, the most
280 dominant OHPhe isomer from all sites was 1-9-OHPhe, which is similar to results obtained
281 by Fan et al. (2012) in humans. However, in Kokote, 4-OHPhe was most abundant (Table
282 3). In human urine, Thai et al. (2016) and Levine et al. (2015) found 1-OHPhe as most
283 dominant while Guo et al. 2013 also reported 3-OHPhe as the most dominant of four
284 phenanthrene metabolites. These variations could be due to differences in metabolic
285 pathway among species. The possible source of phenanthrene and fluorene exposure to
286 cattle in Kokote could be due to inhalation during combustion at low temperatures such as
287 wood or grass combustion since the low molecular weight, LMW (< 4 rings) PAH
288 compounds are abundant during low temperature combustion (Lake et al., 1979). Because
289 Kokote is mainly agricultural area with many farmlands, resident farmers frequently
290 practice bush burning. Another possible source could be due to ingestion of soil or water
291 since the cattle graze freely and the soil from which they pick food or water may be bound
292 to PAHs from burning activities. Previous study by Tay and Biney (2013) indicated that
293 agricultural soils in Accra, Ghana were dominated by LMW PAHs through which domestic
294 animals could be exposed. PAHs tend to adsorb tightly to organic matter in soil rendering
295 them less susceptible to biological and chemical degradation (Hatzinger et al., 1997) and in
296 general, LMW PAHs are more water soluble than HMW PAHs (Nam et al., 2008).

297 *3.5. Association between urinary OH-PAHs concentrations and sex*

298 Gender differences have been used in various studies to predict differences in OH-PAHs
299 concentrations in human (Sul et al. 2012; Levine et al., 2015; Bartolomé et al., 2015; CDC,
300 2015). Study by Thai et al. (2016) in human urine showed no association between sex and

301 urinary OH-PAHs concentrations. In this study, 2-OHNap was significantly higher ($p <$
302 0.05) in male cattle (GM_{SG} = 4.43 ± 7.16 ng/mL) compared to females (GM_{SG} = 2.01 ±
303 5.12 ng/mL) (Table 4). Kim et al. (2013) suggested that rates of intake, accumulation, and
304 excretion of chemicals differ in male and female cattle, although ADME (absorption,
305 distribution, metabolism, and excretion) data would be needed to support that assertion.
306 Differences could also be due to different rearing systems from the various sites.

307 Several non pharmacogenetic factors such as age, gender, species, disease factors or
308 exposure to environmental pollutants might contribute to the expression and regulation of
309 hepatic P450 in man, laboratory species and domestic animals (Guengerich, 2002; Nebbia,
310 2001). In a study by Dacasto et al. (2005), male piedmontese cattle showed significantly
311 higher CYP3A-dependent drug metabolizing enzymes, erythromycin *N*-demethylase
312 (ERDEM), ethylmorphine *N*-demethylation (ETDEM) and testosterone 6ß-hydroxylation
313 (6ß-OHT), activities compared to females, with the exception of testosterone 2ß-
314 hydroxylase, 2ß-OHT, (whose enzymatic activity was yet lower in females). On the other
315 hand, no gender-difference was noticed in limousin cattle.

316 In human, Sul et al. (2012) observed significantly higher levels of urinary 2-OHNap in
317 men than females, and suggested that gender were predictors of urinary 2-OHNap
318 concentrations. 1-9-OHPhe on the other hand was significantly higher in female cattle
319 (GM_{SG} = 2.17 ± 1.18 ng/mL) than males (GM_{SG} = 1.71 ± 1.07 ng/mL) (Table 4), while
320 ∑OHPhes in women were low compared to men (Bartolomé et al., 2015). These differences
321 could be due to variations in metabolism, levels and route of exposure to PAHs. The
322 urinary levels of 1-OHNap, 2-OHNap, ∑OHNap, 1-OHPhe, 9-OHPhe, ∑OHPhe, 1-OHPyr,

and ∑OHPAHs among women were all significantly or marginally higher than those among men workers (Guo et al. 2014). In consistency, Guo et al. (2014) found that, when exposed to similar levels of PAHs, women had significantly higher micronuclei frequencies than men. Emerging evidence also indicates that women may be at greater risk of lung cancer than men, probably 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).

4. Conclusions

Cattle urine samples were collected from both urban (Kumasi) and rural (Offinso) sites in the Ashanti Region, Ghana, and GM_{SG} concentration of OH-PAHs indicated that, 2-OHNap was the most abundant followed by; 1-9-OHPhe > 4-OHPhe > 1-OHPyr > 2-3OHFlu > 2-OHPhe > 3-OHPhe. The results of the present study showed that cattle in Kokote (rural area) were exposed to significantly higher levels of PAHs than the other sites, and naphthalene-containing-mothballs might have contributed significantly to 2-OHNap levels detected in cattle urine. There was no significant difference between urinary OH-PAHs concentrations in cattle in urban and rural sites except for 2-OHPhe and 4-OHPhe and similar to urban areas, rural sites could also be polluted with PAHs. Levels of 2-OHNap was significantly higher in male cattle compared to females, while the opposite was for 1-9-OHPhe.

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Figure captions:

Fig. 1 Map showing cattle urine sampling locations in the Ashanti Region, Ghana (yellow pins indicate sampled locations and red pin indicate city centre in Kumasi)

Fig. 2 Geometric mean concentrations (adjusted by specific gravity) of OH-PAHs in cattle urine from 5 sample sites in Kumasi and Offinso, Ghana

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571 Table 1: Quality assurance and control (QA/QC) for OH-PAHs analysis in cattle urine

Compound name	LOD (ng/mL)	LOQ (ng/mL)	ISTD Recovery (%)	Spiked solvent blanks (%)	Matrix spikes (%)
2-OHNaphthelene	0.0898	0.295			
9-OHFluorene	0.181	0.603			
2-3-OHFluorene	0.0745	0.248			
13C6-2-OHFluorene			94	96	96
1-9-OHPhenanthrene	0.214	0.714			
2-OHPhenanthrene	0.0696	0.232			
3-OHPhenanthrene	0.348	1.16			
4-OHPhenanthrene	0.0437	0.145			
3-OHPhenanthrene-d9			96	98	95
1-OHPyrene	0.259	0.865			
13C6-1-OHPyrene			89	92	85
6-OHChrysene	0.220	0.733			
3-OHBenzo(e)Pyrene	0.162	0.542			
9-OHBenzo(a)Pyrene	0.0959	0.319			

572 Italicized compounds are internal standards (ISTD); LOD: Limit of detection; LOQ: Limit
573 of quantification

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Table 2: Specific gravity adjusted urinary OH-PAHs concentrations (ng/mL) in cattle from urban and rural sites in Kumasi and Offinso, Ghana

Site	n	2-OHNap (GM_{SG} ± SD)	2-3-OHFlu (GM_{SG} ± SD)	2-OHPhe (GM_{SG} ± SD)	1-9-OHPhe (GM_{SG} ± SD)	4-OHPhe (GM_{SG} ± SD)	1-OHPyr (GM_{SG} ± SD)
Urban	17	2.29 ± 3.40^{a}	0.919 ± 0.462^{a}	0.245 ± 0.171^{a}	1.96 ± 1.14^{a}	1.09 ± 0.841^{a}	1.52 ± 0.873^{a}
Rural	78	2.91 ± 6.37^{a}	1.24 ± 1.97^{a}	0.552 ± 0.598^{b}	2.03 ± 1.17^{a}	1.88 ± 2.01^{b}	1.27 ± 0.824^{a}

n: number of samples; GM_{SG}: geometric mean concentration adjusted by specific gravity; SD: standard deviation; different letters (a and b) within a column indicate significant differences (Student's T-Test; $p < 0.05$)

Table 3: Specific gravity adjusted OH-PAHs concentrations (ng/mL) in cattle urine

Sample site	n	Location	2-OHNap (GM_{SG} ± SD)	2-3-OHFlu (GM_{SG} ± SD)	1-9-OHPhe (GM_{SG} ± SD)	2-OHPhe (GM_{SG} ± SD)	3-OHPhe (GM_{SG} ± SD)	4-OHPhe (GM_{SG} ± SD)	1-OHPyr (GM_{SG} ± SD)
Oforikrom	8	urban	4.15 ± 4.37^{b}	0.99 ± 0.62^{b}	1.67 ± 1.21^{b}	0.17 ± 0.10^{c}	nd	0.73 ± 0.41^{c}	1.37 ± 1.18^{ab}
Santasi	9	urban	0.61 ± 0.23^{cd}	0.75 ± 0.35^{b}	2.26 ± 1.14^{ab}	0.32 ± 0.20^{bc}	nd	1.14 ± 0.33^{bc}	1.33 ± 0.80^{ab}
Twumasen Estate	31	rural	0.69 ± 0.57^{d}	0.31 ± 0.13^{c}	1.73 ± 0.58^{ab}	0.29 ± 0.26^{c}	nd	1.50 ± 0.86^{b}	0.99 ± 0.73^{b}
Saboa	40	rural	1.24 ± 0.67^{c}	0.80 ± 0.79^{b}	2.07 ± 1.43^{ab}	0.41 ± 0.16^{b}	nd	1.27 ± 0.91^{bc}	1.16 ± 0.74^{b}
Kokote	7	rural	21.9 ± 6.51^{a}	6.74 ± 1.41^{a}	3.12 ± 0.79^{a}	2.26 ± 0.51^{a}	2.1±0.57	7.49 ± 1.73^{a}	2.29 ± 1.28^{a}

n: number of samples; nd: below limits of quantification (LOQ); different letter (a, b, c and d) within a column indicate significant difference ($p < 0.05$) among communities; GM_{SG}: geometric mean concentration adjusted by specific gravity; SD: standard deviation

Table 4: Specific gravity adjusted urinary OH-PAHs concentrations (ng/mL) in male and female cattle in Kumasi and Offinso, Ghana

Sex	2-OHNap (GM_{SG} ± SD)	2-3-OHFlu (GM_{SG} ± SD)	1-9-OHPhe (GM_{SG} ± SD)	2-OHPhe (GM_{SG} ± SD)	3-OHPhe (GM_{SG} ± SD)	4-OHPhe (GM_{SG} ± SD)	1-OHPyr (GM_{SG} ± SD)	∑OHPAHs (GM_{SG} ± SD)
Male	4.43 ± 7.16^{a}	1.36 ± 1.94^{a}	1.71 ± 1.07^{a}	0.534 ± 0.650^{a}	0.364 ± 0.658^{a}	1.91 ± 2.22^{a}	1.19 ± 0.981^{a}	11.5 ± 14.6^{a}
Female	2.01 ± 5.11^{b}	0.950 ± 1.65^{a}	2.16 ± 1.18^{b}	0.468 ± 0.510^{a}	0.237 ± 0.499^{a}	1.65 ± 1.69^{a}	1.22 ± 0.827^{a}	8.71 ± 10.0^{a}

GM_{SG}: geometric mean concentration adjusted by specific gravity; SD: standard deviation; ∑OHPAHs: sum of OHPAHs; different letters (a and b) within a column indicate significant differences (Student's T-Test; $p < 0.05$)

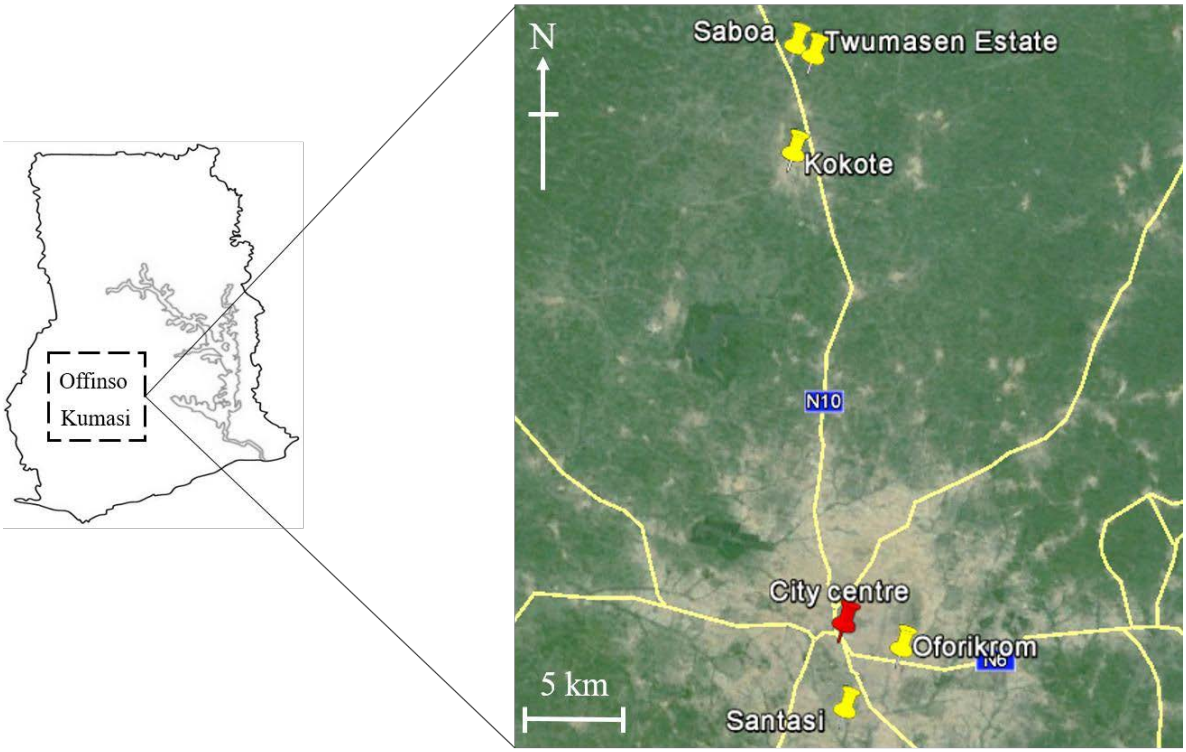
Offinso
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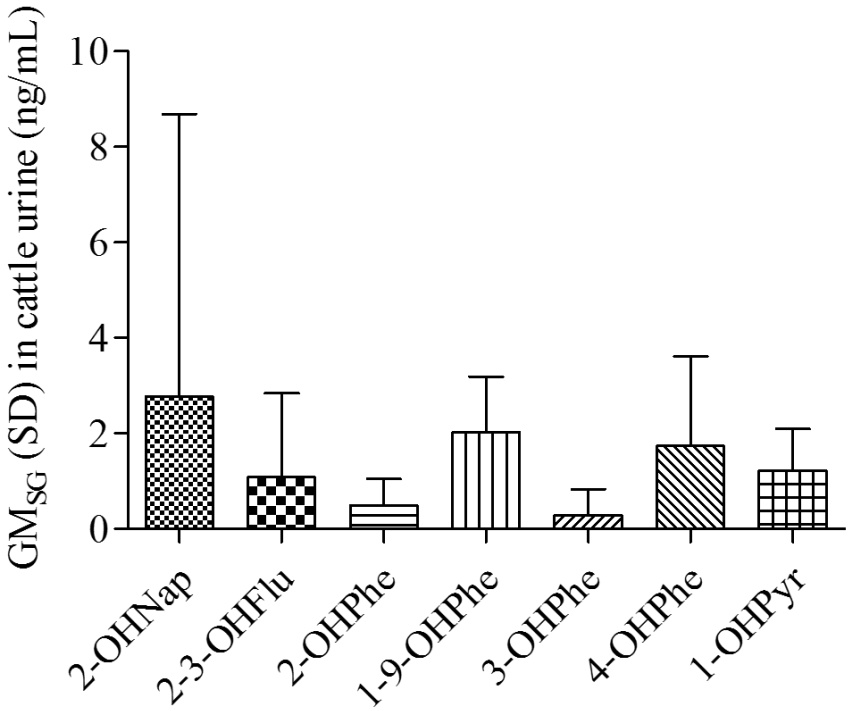
Fig. 1


GMSG (SD) in cattle urine (ng/mL)
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0
2-OHNap
2-3-OHFlu
2-OHPhe
1-9-OHPhe
3-OHPhe
4-OHPhe
1-OHPyr

Fig. 2