Background Levels of Dioxin-like Polychlorinated Biphenyls (dlPCBs), Polychlorinated, Polybrominated and Mixed Halogenated Dibenzo-p-dioxins and Dibenzofurans (PCDD/Fs, PBDD/Fs & PXDD/Fs) in sera of Pregnant Women in Accra, Ghana.

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Abstract

Human exposure data on dioxins and dioxin-like compounds (DLCs) in Ghana are limited. Based on health risks associated with dioxins and DLCs, the impact of maternal body burdens on foetal exposure is significant. This is the first study that assesses polychlorinated, polybrominated and mixed halogenated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs, PBDD/Fs and PXDD/Fs), and dioxin-like polychlorinated biphenyls (dlPCBs) in sera of primiparous Ghanaians. Our sample selection includes 34 participants from two municipalities (Accra and Tema), and explores contributions from environmental and dietary exposures using questionnaire data. Sample preparation involved C_{18} solid phase extraction, purification with acidified silica and lipid removal cartridges, and detection with gas chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. The calculated average toxic equivalent concentration was 5.3 pg TEQ/g lw, with contributions from dlPCBs (1.25 pg TEQ/g lw), PCDD/Fs (3.10 pg TEQ/g lw), PBDD/Fs (0.49 pg TEQ/g lw) and PXDD/Fs (0.50 pg TEQ/g lw). The calculated total TEQ concentration was lower than background TEQ concentrations reported in sera of pregnant women globally. Positive correlations were obtained for total dioxins and DLC concentrations with age and Body Mass Index (BMI). Dietary intake of seafood and dairy products had a strong influence on PCDD/F and dlPCB concentrations. Statistically significant differences were observed for dioxins and DLCs in participants from Accra (in close proximity to Agbobloshie e-waste site) and Tema. Given the significant TEQ contribution of PBDD/Fs and PXDD/Fs (~20%), it is essential to explore these classes of dioxins and DLCs in future biomonitoring studies as they may pose health risks, and add extra diagnostic information in source exposure investigations.

Keywords: Ghana; background concentrations; dioxins; dioxin-like compounds; serum; pregnant women

1.0 Introduction

Dioxins and dioxin-like compounds (DLCs) are a class of persistent organic pollutants (POPs) produced from combustion and industrial processes of brominated and/or chlorinated organic compounds. The most toxic dioxins and DLCs include 12 polychlorinated biphenyls (dioxin-like PCBs- dlPCBs), and 17 congeners of 2378-polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) (Van den Berg et al., 2006). Structurally related analogues- 2378-polybrominated and mixed halogenated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs and PXDD/Fs, X=Br and Cl) exhibit similar toxicity profiles (Birnbaum et al., 2003; Olsman et al., 2007; Van den Berg et al., 2006). This class of compounds bioaccumulate in the environment; in humans, they bind with the aryl hydrocarbon receptor (AhR) and induce toxic effects (Mason et al., 1987; Olsman et al., 2007).

For many decades, occupational, accidental (unintentional) and background exposure to dioxins and DLCs have resulted in adverse health concerns including carcinogenic and dermal defects, neurodevelopmental and reproductive health effects (Arisawa et al., 2005; Hites, 2011; White and Birnbaum, 2009). Populations occupationally or accidentally exposed to POPs usually possess higher body burdens. For background exposure populations, concentrations are lower, can be variable for diverse cultural groups, vulnerable groups, and can also be impacted by age and gender (Porta et al., 2008). Approximately 90% of human background exposure to dioxins and DLCs arise from dietary intake of contaminated food (Djien Liem et al., 2000). However, questions about critical windows of foetal and early-life infant exposure to dioxins and DLCs, and their impacts on embryonic, infant developmental or later life consequences, reflect on placental transfer of dioxins and DLCs in nutrients from maternal blood and breastmilk during infancy (Caspersen et al., 2016; Needham et al., 2010; Pryor et al., 2000; Schecter et al., 2006). Studies undertaken on maternal participants, have

highlighted epidemiological evidence of short and long-term effects of in-utero exposure. These include associations between maternal exposure, maternal diet, maternal age, hormonal disruptions in children, and impacts on estrogenic metabolism (Baba et al., 2018; Cao et al., 2008; Lignell et al., 2016; Miyashita et al., 2018; Nakajima et al., 2017; Papadopoulou et al., 2014; Wittsiepe et al., 2008). Additionally, investigative studies on foetal exposure to dioxins and DLCs provide evidence of an association between maternal serum with foetal abortion, birth defects and low birth weight (Guo et al., 1995; Nham Tuyet and Johansson, 2001; Yamashita and Hayashi, 1985). Although higher concentrations of dioxins and DLCs have been detected in placenta (Schecter et al., 1990; Schecter et al., 1996; Suzuki et al., 2005; Wang et al., 2004), and umbilical cord blood (Koopman-Esseboom et al., 1994; Suzuki et al., 2005), venous blood sample during pregnancy is considered the most representative to evaluate maternal or foetal body burdens.

In the last decade, numerous studies have supported increasing evidence of adverse effects of dioxins and DLCs in general and occupationally exposed populations (Fromme et al., 2009; Hong et al., 2009; Mato et al., 2007; Patterson et al., 2008; Patterson et al., 2009). Only a few studies on dioxins and DLCs have been documented in African populations. These include populations in Egypt, South Africa, Morocco, Canary Island- Spain and Ghana (Adu-Kumi et al., 2010b; Asante et al., 2011; Henríquez-Hernández et al., 2016a; Henríquez-Hernández et al., 2016b; Luzardo et al., 2014; Pieters and Focant, 2014; van den Berg et al., 2017; Wittsiepe et al., 2015). Records of non-dlPCBs have been reported in sera and breastmilk (Asamoah et al., 2018; Asante et al., 2011; Darnerud et al., 2011; Ennaceur and Driss, 2010; Hassine et al., 2012; Müller et al., 2017; Röllin et al., 2009) in some African countries.

Ghana is located along the southernmost part of the West coast of Africa, and has a wide range of potential sources of POPs. These include a historical legacy of pesticide use, along with more recent sources of emerging pollutants such as dioxins and DLCs from the electronic waste site in Agbogbloshie. To date, the current knowledge on POPs in Ghana dominantly relates to the presence of organochlorine pesticides (OCPs) and non-dlPCBs in environmental matrices. A review on the state of POPs in Ghana emphasized concerns of an absence of human biomonitoring studies on dioxins, DLCs and emerging contaminants (Bruce-Vanderpuije et al., 2019a). The limited studies on human biomonitoring, undertaken in Ghana, have identified health risks from infant dietary intake of OCPs and non-dlPCBs in breastmilk in occupationally and non-occupationally exposed lactating mothers in farming, fishing and e-waste communities in Ghana (Asamoah et al., 2018; Asante et al., 2011; Asante et al., 2013; Ntow, 2001; Ntow et al., 2008). Pioneering studies on dioxins and DLCs in soil from e-waste sites, sera of e-waste workers, breastmilk from lactating mothers, and edible lake fish in Ghana point to possibilities of bioaccumulation in humans (Adu-Kumi et al., 2010a; Adu-Kumi et al., 2010b; Tue et al., 2016; van den Berg et al., 2017; Wittsiepe et al., 2015). Two studies have quantified infant exposure to PCDD/Fs and dlPCBs in milk from Ghanaian lactating mothers- The Ghana Environmental Protection Agency, and a global survey completed by the World Health Organization (WHO)/United Nations Environment Programme (UNEP) (Adu-Kumi et al., 2010b; van den Berg et al., 2017). There are currently no background exposure studies that have quantified the toxic equivalents, and/or assessed the risks of exposure of pregnant women and foetuses to one of the most toxic class of POPs- dioxins and DLCs in sera of pregnant women in Ghana. Based on health concerns of mother and foetus, the goal of this study is to provide baseline concentration data and to quantify the background exposure levels of 17 congeners of PCDD/Fs, 12 congeners of dlPCBs, 7 congeners of 2378-substituted PBDD/Fs and 7 congeners of 2378-substituted PXDD/Fs in 34 primiparous Ghanaians.

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142 1.1 Study sites

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144 Samples were obtained from participants in Tema and Accra, two municipalities
145 located along the South coast of Ghana within the Greater Accra region. These sites are noted
146 to generate industrial pollution including organic pollutants (releases into air and water bodies)
147 from local manufacturing industries. In addition, Agbogbloshie e-waste site, situated in
148 industrial area in Accra, is noted for the release of organic pollutants from open-air burning of
149 scrap electronic waste. Figure 1 shows a description of the two study sites. Residential homes
150 of pregnant women (participants) were at average distances of 5.6 miles (Accra) and 12 miles
151 (Tema) away from the Agbogbloshie e-waste site.

152 It is important to note that participants in this study had no known occupational or
153 accidental exposure. This study provides important baseline data for background
154 concentrations of dioxins and DLCs within primiparous Ghanaians. The study further aims to
155 identify the differences in exposure levels to dioxins and DLCs within the two municipalities,
156 and to identify linkages with dietary patterns or local sources of pollution. A comparison of
157 findings from this study with existing global datasets for similar cohorts were made on
158 calculated toxic equivalent concentrations (TEQs) for PCDD/Fs and dlPCBs using the
159 WHO_{2005}-Toxic Equivalency Factors (TEFs). Concentrations of PBDD/Fs and PXDD/Fs were
160 also quantified and the tentative TEQs calculated for the brominated and mixed halogenated
161 analytes were based on TEFs from their corresponding chlorinated analogues. In addition, other
162 types of PXDD/F and PCB analytes that are not considered as toxic as 2378-substituted dioxins
163 and DLCs were quantified, when identified.

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2.0 Material and Methods

2.1 Participant Recruitment

The study was approved by the Ghana Health Service Ethics Review Committee, and conducted in accordance with ethical principles for medical research involving human subjects. Women in their eighth month of pregnancy were interviewed by research nurses during routine health check-ups at the clinics. Participants recruited from The Greater Accra Regional and Tema General Hospitals, voluntarily completed informed written consent forms and exposure assessment questionnaires prior to sample collection. Data on age, occupation, diet, cigarette smoking and alcohol intake and bodyweight were documented. Maternal venous blood (15 mL, n = 34) was collected in April 2017, from primiparous women in Accra (n = 17) and Tema (n = 17). Blood was collected into clear 15 mL Corning centrifuge tubes without anticoagulant; 5-6 mL serum was obtained by centrifugation in an Eppendorf Centrifuge 5810 at 4000 rpm/rcf for 10 min, within 24 hours of sample collection. Serum samples were stored at -20 ^{o}C prior to extraction and analysis.

2.2 Reagents and Chemicals

Distilled in glass grade organic solvents- n-hexane, toluene, nonane, acetonitrile, methanol and water were obtained from Caledon Laboratories Limited (Georgetown, Ontario, Canada). Formic acid, 88% analytical grade reagent and octadecyl non-endcapped bonded silica C_{18} cartridges (500 mg/6 mL) were obtained from Thermo Fisher Scientific. Captiva EMR-Lipid removal cartridges (600 mg/6 mL) were obtained from Agilent Technologies. For the separation of planar and co-planar PCDD/Fs, dlPCBs and PXDD/Fs (and PBDD/Fs) from non-planar compounds, ultra clean carbon mini-columns (2%) and re-usable glass column reservoirs (20 cm in length, 0.5 cm in diameter) from Cape Technologies were used. ^{13}C-

labelled isotope and native PCDD/Fs, dlPCBs and PXDD/Fs standards were obtained from Wellington Laboratories Inc. (Guelph, Ontario, Canada). Chromatographic separation column: DB5-MS (5% diphenyl 95% dimethyl polysiloxane, 60 m x 0.25 mm ID x 10 μm film thickness, J&W Scientific, CA, USA) was obtained from Agilent.

Preparation of calibration, recovery and injection standards for PCDD/Fs, PXDD/Fs, PBDD/Fs and dlPCBs are described in detail in Section 1.2 in the Supplementary information. PCDD/F, PBDD/F, dlPCB, and PXDD/F analytes analysed in this study are listed in Table S1. All standards were prepared in nonane, except for recovery spiking solutions which were prepared in methanol.

2.3 Sample Extraction

Sample extraction and clean-up steps used in this study were based on analytical procedures from CDC with modifications (Centre for Diseases Control and Prevention, 2016). Extraction was performed on serum samples using C_{18} solid phase extraction (SPE) with hexane, clean-up using Captiva-EMR lipid removal cartridge and acidified silica, and fractionation on carbon column. Samples were analysed using capillary gas chromatography with atmospheric pressure chemical ionization (APCI) and a triple quadrupole mass spectrometer (GC-APCI-MS/MS, Xevo TQ-XS) from Waters Corporation, Manchester, UK.

The analytical procedure for method development is detailed in Section 1.3 in the Supplementary information. For sample extraction, briefly, 2 g of serum was spiked with 5 μL of 2 pg/μL label recovery mix- $^{13}C_{12}$-PCDD/Fs, $^{13}C_{12}$-dlPCBs and $^{13}C_{12}$-PXDD/Fs- to determine extraction efficiency, matrix effects on recovery and enable quantitation by isotope

dilution mass spectrometry. Two millilitres of water was added for matrix dilution; 2 mL formic acid for protein denaturation. Serum sample mix was vortexed and sonicated for 15 min in between additions. C_{18} cartridges were conditioned gravimetrically using two cartridge volumes of methanol and water prior to loading serum mixture, and eluted at a flow rate of 0.6 mL/min. Serum culture tubes were rinsed with 2 x 5 mL H_2O and transferred onto C_{18} cartridge barrels. Cartridges were dried under vacuum pump suction for 1 hour to remove water. Analytes were eluted from C_{18} cartridge using 3 x 5 mL hexane and collected in clear EPA vials, at a flow rate of 0.6 mL/min.

2.4 Lipid removal Clean-up and Fractionation

Extracts were evaporated to 500 µL; 3 mL acetonitrile was added. Extracts were loaded onto Captiva-EMR Lipid removal cartridge and allowed to flow under gravity. Vials were rinsed with 5 mL ACN and loaded onto EMR cartridge. Eluate was collected under gravity into EPA vials. Extracts were evaporated to 1 mL.

For each sample, acidified silica cartridge was connected to a carbon column and activated with 20 mL hexane. A 1 mL serum extract was loaded and the cartridge was rinsed with 30 mL hexane. The acidified silica cartridge was replaced with a reusable glass column reservoir. The carbon column was then inverted and eluted with 30 mL toluene. Eluate was collected in 40 mL EPA vials, evaporated to 350 µL under low N_2, transferred into inset vials, evaporated to dryness, and reconstituted with 10 µL of 1 pg/µL injection standard. A flow chart of sample preparation steps is shown in Figure S1.

2.5 Lipid measurement

Five-hundred microlitres of each serum sample was used to determine the total lipid concentration. Enzymatic analysis of cholesterol and triglycerides was completed using a calibrated BT3000 chemistry auto analyzer (Biotechnica Instruments). Total lipids were calculated from the sum of the total cholesterol and triglycerides concentrations using the formula:

$$\text{Total lipids} = (2.27 \times \text{Total cholesterol}) + \text{Triglycerides} + 62.3 \text{ mg/dL} \quad \text{Equation 1}$$

Where total lipids, total cholesterol, triglycerides concentrations are reported as mg/dL.

2.6 Instrumental Analysis

The gold standard for measuring trace levels (femtogram) of POPs including PCDD/Fs has traditionally been with a gas chromatograph coupled to a double focusing magnetic deflection (sector)-high resolution mass spectrometer (GC-HRMS). However, gas chromatography with atmospheric pressure chemical ionization and tandem mass spectrometry (GC-APCI-MS/MS) has recently been used successfully to match the performance of the sector instrument for the analysis of PCBs, and PCDD/Fs (García-Bermejo et al., 2015; Geng et al., 2016; Megson et al., 2016; Organtini et al., 2015a; Organtini et al., 2015b; Stubleski et al., 2018; van Bavel et al., 2015). Sample analyses was performed using GC-APCI-MS/MS. Method development was performed on a Q-Ion Mobility Spectrometry-ToF instrument (APGC Synapt G2-Si) operating in full scan mode. A 1 µL sample extract was injected on a DB5-MS (60m x 0.25mm x 0.1µm) non-polar stationary phase column. Instrumental parameters and operating conditions are summarized in Table S2. The mass spectrometer was operated in positive ion mode, using multiple reaction monitoring. Four transitions (2

quantifiers and 2 qualifiers) were monitored for native and $^{13}C_{12}$ labelled dlPCBs, PCDD/Fs and PXDD/Fs. For PCDD/Fs the loss of -COCl was monitored (Table S1a). For dlPCBs, the loss of Cl_2 was monitored (Table S1b). Based on studies of Organtini et al. (2015a, b) and Myers et al. (2012), and from the results of the method development, ions monitored for mixed halogenated dibenzo-p-dioxins and dibenzofurans included native and label -COBrCl, -COBr, -$(CO)_2$BrCl, -COCl, -Br_2, and -Br (Myers et al., 2012; Organtini et al., 2015a; Organtini et al., 2015b). The transitions, collision energies and isotope ratios are summarized in Table S1.

2.6.1 **Quality assurance/ Quality Control**

Analytes were quantified by isotope dilution using ions specified in Table S1. For non 2378-PXDD/Fs and PBDD/Fs for which there were no ^{13}C labeled standards, a semi-quantitative method was utilized in quantification. A detailed description on the methodology for reporting linearity, quality control and method detection limits are explained in Section 1.5 in the Supplementary Information. For linearity, the response obtained for a native, relative to its corresponding label ^{13}C standard was linear for the range of calibration standards analyzed. Calculated coefficient of determination for PCDD/F analytes was $R^2 \geq 0.998$ (except for OCDD and OCDF: $R^2 = 0.984$), that for dlPCBs and PXDD/Fs were $R^2 \geq 0.995$ and $R^2 \geq 0.996$, respectively. The percentage RSDs obtained for PCDD/Fs, dlPCBs and PXDD/Fs ranged between 1.6 and 13.8%; this is in agreement with the acceptable 15% value (Centre for Diseases Control and Prevention, 2006). Method validation was performed by analysis of fortified serum NIST Standard Reference Material (SRM) 1958. Recoveries for NIST standard ranged between 75% and 105% for PCDD/Fs, and 67.5% and 96.3% for dlPCBs. The results for the SRM are presented in Supplementary Information-Section 1.3; data is presented in Table S3a and 3b. Recoveries for isotopically labelled standards, spiked into serum prior to

extraction/clean-up, ranged between 47.9 and 120% for dlPCBs, 47.3 and 129.9% for PXDD/Fs, and 41.8 and 140% for PCDD/Fs (recoveries obtained fell within the acceptable EPA ranges (40-145%) (EPA, 2010), except for 4 samples, for which ^{13}C-OCDD and ^{13}C-1,2,3,4,6,7,8-HpCDD ranged between 28.8 and 37%). The instrument limit of detection (iLOD) was restricted by the lowest detectable standard concentration, and ranged between 5 and 100 fg/µL for PCDD/Fs and PXDD/Fs, and 5 fg/µL for dlPCBs. Where concentrations were below the LOD, ½ LODs were assigned and used in the TEQ calculations.

The lipid adjusted sample serum concentrations of dioxins and DLCs are reported as pg/g lipid. The Toxic Equivalent (TEQ) for each class of dioxins and DLCs was reported as pg WHO-TEQ/g lipid weight (lw)(Van den Berg et al., 2006).

2.6.2 Statistical Analyses

Dioxins and DLCs in sera were statistically analysed using the Minitab 18 software package (Minitab, 2010), to determine differences in concentrations for the three groups of analytes: PCDD/Fs, dlPCBs and PXDD/Fs. Spearman rank correlation and bivariate linear regression were used to evaluate bivariate associative correlations between dioxins and DLC concentrations, and factors such as age, gestational week, food consumed and body mass index (BMI), and congener concentrations of PCDD/Fs, PXDD/Fs and dlPCBs, as well as total concentration (PCDD/Fs + PXDD/Fs + dlPCBs). Multivariate statistical analysis (JMP®, Version *14.1.* SAS Institute Inc., Cary, NC, 1989-2007) was used to assess congener-specific distributions. Exploratory data analysis was conducted using Principal Component Analysis (PCA) with Hierarchical Cluster Analysis (HCA) using both box-cox normalized and log-normalized contaminant concentrations. This was performed to investigate if participants with

315 different characteristics (e.g. different geographical areas, dietary preferences and age) had a
316 distinctive chemical pattern.
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318 The normality of data distribution was checked with Kolmogorov-Smirnov test. A log-
319 normal distribution for PCDD/F, PXDD/F and dlPCB concentrations for 34 pregnant women
320 dataset was identified. Because the serum concentration data was not normally distributed,
321 descriptive statistics for central tendency of the data was based on the geometric mean rather
322 than on arithmetic mean. The range and 95% confidence interval were used to describe the
323 data. In addition, the relative percentages of each congener to the total concentration was
324 evaluated for each class of dioxins and DLCs.
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3.0 Results and Discussion

3.1 Food consumption

Questionnaire responses on food consumption included major dietary intake of seafood, fish, meat and meat products, and dairy products; this can be located in Supplementary Information- Section 1.7. Seafood consumed included shrimps, clams, mussels, snails, squid, oysters, and lobsters. Fish types included: salmon, mackerel, tilapia, tuna, and dried herring. Dairy products frequently consumed included eggs and milk. Majority of the participants reported consumption patterns that were generally similar for both Tema and Accra municipalities.

3.2 Characteristics of pregnant women

The characteristics of the participants are presented in Table 1. All participants were primiparous non-smokers; the mean age was 25 yrs (range: 18-38 yrs). The mean gestational age was 31.9 weeks, and the average body weight was 75.7 kg. Almost 40% of participants had a BMI that exceeded 25 kg/m^2 (overweight), none was underweight, and 60% had healthy weight (range: 21.3-24.9 kg/m^2). Fifty percent (17) of the subjects resided in Tema, and 50% (17) in Accra, Ghana.

3.3 Congener specific dioxins and DLCs identified

PCBs 105 and 118 were detected in all the participants. The remaining congeners (114, 126, 156, 157, 169 and 189) were regularly detected in participants with detection frequencies of 82.4 and 97.6%. For PCDD/Fs, analytes for which serum concentrations were consistently

362 above the LOD were 12378-PeCDF, 23478-PeCDF, 123478-HxCDF, 1234678-HpCDF,
363 OCDF, 123478-HxCDD, 123678-HxCDD, 123789-HxCDD, 1234678-HpCDD and OCDD.
364 Concentrations of chlorinated dioxins and PCBs have been widely reported in human serum;
365 however, much less congener specific data has been provided for mixed halogenated dioxins
366 and furans. This study provides an important baseline data showing that 8-B-234-CDF, 3-B-
367 278-CDF, 12-B-78-CDF and 4-B-2378-CDF were consistently identified in all 34 participants.
368 Seven congeners of 2378-PXDD/F and 7 congeners of 2378-PBDD/F were detected
369 intermittently. Concentrations of non-2378-PBDD/Fs and PXDD/Fs for 8-B-23-CDF, 7-B-23-
370 CDD, 2-B-78-CDD, 2-B-378-CDD, 23-B-78-CDF, 13-B-278-CDF, 1378-BDD, 2378-BDF,
371 1234-BDD, 12478-BDD and 23478-BDF were all below the LOD.
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373 **3.4 Concentrations of dioxins and DLCs**
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375 The baseline data obtained for both localities are presented in Table 2. The
376 concentrations of dioxins and DLCs in participants were within one or two orders of magnitude
377 where dlPCBs > PCDD/Fs > PBDD/Fs and PXDD/Fs. The total mean concentrations for
378 dlPCBs, PCDD/Fs, and PBDD/Fs (and PXDD/Fs) in Tema were: 59.3 pg/g lw, 52.6 pg/g lw,
379 and 2.47 pg/g lw, respectively. The mean concentrations in Accra were slightly higher than
380 that for Tema for dlPCBs, PCDD/Fs and PBDD/Fs (and PXDD/Fs); these were 96.1 pg/g lw,
381 71.8 pg/g lw, and 4.19 pg/g lw, respectively. Statistically significant differences were observed
382 between mean concentrations of dlPCBs in Tema and Accra with the exception of PCB 157
383 (p-value = 0.092). For PCDD/Fs, statistically significant differences were observed for 4 furan
384 congeners: 2378-TCDF, 1234678-HpCDF, 1234789-HpCDF and OCDF, for both groups of
385 participants. Concentrations of 7 of the 14 congeners of 2378-PXDD/Fs and PBDD/Fs (2-B-
386 378-CDD, 23-B-78-CDF, 23-B-78-CDD, 13-B-278-CDF, 2378-BDF, 12378-BDF, 12378-

BDD), were also statistically significantly higher in participants from Accra than Tema. Both sites- Accra and Tema are along the coastal areas; however, the differences observed in dlPCBs and the 4 sets of congeners may be explained by subtle dietary patterns as different food groups have been shown to have an important influence on dlPCB and PCDD/F exposure. Other possible reasons for the observed differences could be due to local factors such as combustion processes from exposure sites: Agbogbloshie e-waste site. Further research is required to identify contributions from potential sources. Figure 2 presents a comparison of average concentrations for dlPCBs, PCDD/Fs, and PBDD/Fs (with PXDD/Fs) for the two sites. Tables 2a, 2b and 2c show the baseline data obtained for the two sites for dlPCBs, PCDD/Fs, and PBDD/Fs (and PXDD/Fs) respectively. It also shows p-values obtained from 2-sample t-test (comparison), and their toxic equivalent concentrations (pg TEQ/g lw) for participants from Tema and Accra.

Concentrations of PCB 118, 105 and 156 were significantly higher than other dlPCBs in sera from both Tema and Accra (Table 2a). For PCDD/Fs, in comparison to other congeners detected, 12378-PeCDF, OCDF, 1234678-HpCDD and OCDD were higher in both localities. In addition, contributions from PCDDs were approximately twice that for PCDFs. Similarly, for the sum of congeners of 2378-PBDD/Fs and 2378-PXDD/Fs, a higher total concentration was observed in Accra in comparison to Tema. This could be attributed to greater releases of PBDD/Fs, PXDD/Fs and PCDD/Fs into neighbourhoods from open-air burning of both chlorinated and low molecular weight polybrominated diphenyl ethyl ether (PBDE) containing e-waste materials in Agbobloshie, Accra.

3.5 Total TEQ concentrations

412 Tables 2 and S4 present a summary of the TEQ concentrations of dlPCBs, PCDD/Fs and
413 PBDD/Fs (and PXDD/Fs). The average total TEQ concentrations for participants in Accra and
414 Tema were 6.48 and 4.19 pg TEQ/g lw, respectively. Figure 3 presents a stacked bar chart of
415 TEQ data obtained for the four classes of dioxins and DLCs (dlPCBs, PCDD/Fs, PBDD/Fs and
416 PXDD/Fs) in each participant. Although PXDD/Fs (and PBDD/Fs) were generally present at
417 lower concentrations than for other dioxins and DLCs, they had a large impact on the overall
418 TEQ, accounting for approximately 20% of the total TEQ contribution in participants from
419 both Accra and Tema. This indicates the importance of mixed halogenated dioxins and furans
420 when undertaking human health risk assessments. The largest contributor to dlPCBs TEQ was
421 PCB 126 (the most toxic dlPCB) which accounted for approximately 25%. Large contributions
422 were also recorded for 12378-PeCDD (~25%) and for 2378-TCDD (~20%). The largest
423 contributions from the brominated and mixed halogenated congeners were 2378-BDD (~5%),
424 23-B-78-CDD (~ 4%) and 2-B-1378-CDD (~ 3%). Based on a comparison of relative potencies
425 of PBDD/Fs and PXDD/Fs, 2378-BDF and 3-B-278-CDD are of similar potency as 2378-
426 TCDD (Birnbaum et al., 2003) and this explains their contributions. However, the impact of
427 the TEF influences the congener contribution towards its toxicity; this explains why minimal
428 contributions were observed for OCDD and PCB 118, although these were the dominant
429 congeners detected.

430 To interpret TEQs observed, in a global context, our results were compared with
431 available TEQ data reported in maternal serum in literature (Table 3).

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434 **3.6 Observed relationship between dioxins and DLCs and Ghanaian**
435 **maternal serum**

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To detect associations between age, BMI and dioxins and DLCs, possible effects were assessed from an examination of serum concentration data using Spearman rank correlation and bivariate analysis. An explanation of results is presented in detail in Supplementary Section 1.6. There was statistically no significant difference between residents of Accra and Tema participants in terms of age. From bivariate analysis, the main predictors that confirmed statistically significant associations between mean concentrations of dioxins and DLCs, and total TEQ (PCDD/Fs and dlPCBs) were age and BMI. Additionally, positive correlations confirmed that an increase in BMI potentially induces an increase in dlPCBs, PCDD/Fs and PXDD/Fs during pregnancy. Negative correlations were obtained for the association between gestational week and dioxins and DLCs; an indication that gestational weeks do not necessarily increase dioxins and DLCs. Respectively, the mean concentrations (pg WHO-TEQ/g lw) were highest and lowest for age ranges of 28-38 years and 18-23 years. In overweight pregnant women, mean concentrations of dioxins and DLCs were higher than for pregnant women of normal weight. In addition, dioxin-like exposure was higher in sera of pregnant women who resided in Accra in comparison to those from Tema (Table S4).

3.7 Chemical signatures

3.7.1 Principal Component Analysis (PCA)

Exploratory data analysis involving Principal Component Analysis (PCA) was used to investigate how the relative proportions of dioxins and DLCs changed in different participants. This was performed to determine if participants with different characteristics (e.g. different geographical areas) had a distinct chemical signature. The data set was normalized for dlPCBs, PXDD/Fs and PCDD/Fs by expressing the concentration of each congener as a percentage of the combined sum of congeners within its class. A loading plot was constructed using PCA

(JMP®, Version 14.1. SAS Institute Inc., Cary, NC, 1989-2007) to explain the observed relationships between samples and their contributions. A consideration of all dioxins and DLC data points showed that two demographics described the congener concentrations. Demographic 1 (location), was influenced by PXDD/Fs, and demographic 2 (food type frequently consumed) influenced both PCDD/Fs and dlPCBs. The results indicated that the data was dominated by a few individuals with elevated PCB concentrations. When the data for all dioxins and DLCs were assessed together, it appeared to be of little diagnostic value. However, when certain classes of dioxins and DLCs were removed from the total data set, the geographical locations of the participants were clearly separated. These results showed that PXDD/F congeners were much better at identifying local differences, with PCB profiles also showing some degree of relationship with diet.

3.7.2 PCBs

A plot of the first two principal components shows the variances of normalized concentrations for dlPCBs and PXDD/Fs for the 34 residents of Accra and Tema. Complementary loading plots based on congener profiles that provide similarities in groupings for contaminants observed in the PCA, for participants have also been shown in Figures 4 and 5. The first principal component accounted for 66.9% of the total variability in dlPCB concentrations (Figure 4). These originated from the most abundant dlPCB congeners, and showed a positive correlation towards participants who had lived in Accra during their childhood, irrespective of their current place of habitation (Accra or Tema). The second principal component accounts for 7.5% of the original variance of the data set, and was mostly attributed to dlPCBs, and other non-dlPCBs. Serum samples for residents of Accra were separated from those from Tema by the PCA. Group I consisted of samples R10, R12 and R13 (participants born and raised in Accra), and contained higher proportions of PCBs- 77, 81, 118,

167, 169, 114 and 194. Group II mostly consisted of participants born and raised in other parts of the country who had settled in their matrimonial homes in Accra. These consisted of samples R14-R17, R19 and R2. The concentrations in these samples were enriched with both dlPCBs and non-dlPCBs: PCBs- 128, 206, 118, 105 and 123. The dietary pattern of the group consisted mostly of fish and meat. Group III consisted of participants who had grown up in varying localities of the country and later relocated to either Accra or Tema. These samples consisted of T1-T19, and 9 of the samples from Accra (R1, R3, R5, R6, R8, R9, R11, R15 and R18). The dietary patterns observed mostly consisted of fish, dairy products, seafood and meat. For the majority of these concentrations, the patterns suggest that the source of dlPCBs detected in serum could originate from differences in dietary intake.

3.7.3 PXDD/Fs and PBDD/Fs

Using the Box-Cox normalized concentrations, exploratory data analysis using both PCA and HCA was conducted. Results of the PCA and HCA are shown in Figure 5. For the PCA, two factors accounted for 49.6% (component 1) and 13.3% (component 2) of the variance explained (total 62.9%). Two groups were identified from the PCA clustering. Group I contained 18 participants, some of whom had grown up in poor neighbourhoods in Nima (Accra) and who are currently staying in Accra. From this group, 5 participants possessed a signature that fits the pattern for Accra participants. The major congeners detected in this group were 3-B-278-CDF, 2378-BDF and 1234678-BDD. The potential sources of these congeners include combustion processes from vehicular emissions and biofuel (fuel/charcoal/firewood burning for household and commercial use). In addition, potential sources could further be attributed to the proximity of the Nima locality to the Agbogbloshie e-waste sites. However, as highlighted in a previous study, combustion processes from such a neighbourhood have been identified to contribute to biomass smoke and air-particle pollution in Nima- Accra (Zhou et

al., 2013). Group II consisted of 16 participants, the majority (n =13) of whom grew up in Tema, and still reside in Tema. Commonly detected PXDD/F congeners in this group were 3-B-278-CDF and 2378-BDF and 1234678-BDD. These results indicate the evidence that PBDD/Fs (and PXDD/Fs) are potentially linked to the place of residence of an individual, whereas PCBs are linked to dietary patterns.

3.8 Data comparison with other studies

In the absence of published data on exposure to dioxins and DLCs in sera of primiparous Ghanaians, result comparisons for vulnerable populations with no known exposure cannot be made. However, a comparison between this study and other studies globally (Table 3), shows that the overall mean (5.3 pg WHO-TEQ/g lw) in sera of primiparous Ghanaians is lower than most background concentrations reported in sera of pregnant women in other parts of the world. Results from this study are also lower than median concentrations (PCDDs + PCDFs) detected for occupationally exposed e-waste populations in Ghana: 6.18 pg WHO-TEQ/g_{2005} lw (Wittsiepe et al., 2015); TEQ concentrations detected in the latter (occupationally exposed e-waste workers) are still lower than TEQ concentrations reported in sera of pregnant women globally. Our results [mean total TEQ concentrations [dlPCBs + PCDD/Fs = 4.34 pg TEQ/g lw] are similar to the mean total TEQ concentrations [dlPCBs + PCDD/Fs] detected in individual breastmilk of lactating mothers (n = 42, 6.07 pg TEQ/g lw) in Accra in 2008 in studies of Adu-Kumi et al. (2010b), and from pooled samples from the WHO/UNEP global survey monitoring program (n = 50, 5 pg TEQ/g lw)(Adu-Kumi et al., 2010b; van den Berg et al., 2017). In a similar related study, higher concentrations of the seven indicator PCBs (non-dlPCBs) were detected in breastmilk samples of lactating mothers (occupationally exposed participants and residents: 4.43 ng/g lw) at the Agbogbloshie e-waste

site in comparison to breastmilk samples from Kwabenya (control group: 0.03 ng/g lw), in Accra, Ghana (Asamoah et al., 2018). A high potential health risk estimated from the seven indicator PCBs (including dlPCB 118) indicated significantly higher concentrations in breastmilk from lactating mothers residing in Accra (sum of average PCBs: 82 ng/g lw, PCB 118: 3.0 ng/g lw) in comparison to participants from Kumasi (sum of average PCBs: 65 ng/g lw, PCB 118: 2.6 ng/g lw) and Tamale (sum of average PCBs: 30 ng/g lw, PCB 118: 1.9 ng/g lw). Thus, areas considered to be hotspots in Accra- such as the Agbogbloshie e-waste site and heavy industrial areas can impact background concentrations of dioxins and dioxin-like compounds (Asante et al., 2011).

In order to answer questions on why lower dioxins and DLC concentrations were obtained in comparison to other countries, we considered studies in Ghana that had focused on dietary intake of dioxins and DLCs, since 90% of human background exposure arises from contaminated food (Djien Liem et al., 2000). Due to the absence of publications on estimated daily exposure (dietary intake of PCDD/Fs, and dlPCBs) and exposures to PXDD/Fs and PBDD/Fs for the general population of Ghana, it is not possible to make robust comparisons with other countries. Relatively low concentrations of PCDD/Fs and dlPCBs have been estimated in food (fish) in Ghana, in comparison to other industrialized countries (Adu-Kumi et al., 2010a). Although the consumption of various foods influences the total TEQ for PCDD/Fs and PCBs, there is only one study that has estimated concentrations in tilapia and catfish from Lake Volta in Ghana (and may not necessarily be representative of the entire country). Data/results from the study of Adu-Kumi et al. (2010a), however, serves as a baseline for comparison with other countries. WHO-TEQ_{2005} concentration (wet weight) of PCDD/Fs and dlPCBs in fish from Ghana: ~ 0.3 pg TEQ/g (Adu-Kumi et al., 2010a) was much lower than that estimated in the Baltic Sea: 8 pg TEQ/g- salmon and prat and 12 pg TEQ/g- eel

(Szlinder-Richert et al., 2009), in fresh water or farmed fish from France: ~ 2.8 pg TEQ/g (Tard et al., 2007), and in freshwater fish in South Korea: 1.3 pg TEQ/g (Kim et al., 2004). This indicates that industrialized and developed countries may have a higher dietary intake of contaminated foods- fish, meat, seafood containing PCDD/Fs and dlPCBs in comparison to developing countries such as Ghana.

4.0 Conclusions

To our knowledge, this is the first study on background concentrations of dlPCBs, PCDD/Fs, PBDD/Fs and PXDD/Fs in sera of primiparous Ghanaians. The results provide an average value of 5.3 pg WHO-TEQ/g lw for a cohort of 34 individuals with no known accidental or occupational exposure. This value was generally lower than TEQ concentrations of dioxins and DLCs in other studies of sera from pregnant women in industrialized countries. A breakdown of the results shows that %TEQ contributions in our cohort are predominantly resulting from exposure to PCDD/Fs (57.9%), with significant contributions from dlPCBs (23.4%), as well as PBDD/Fs and PXDD/Fs (18.8%). These percentages suggest that substantial contributions from PCDDs (39.2%) are indicative of sources other than combustion (potentially from dietary exposure). However, contributions from combustion processes (from PBDD/Fs and PXDD/Fs) are also noted to influence the overall TEQ. The results of the PBDD/F and PXDD/Fs data were significantly higher in participants living in close proximity to both e-waste and heavy industrial areas in Accra, than for a group of cohorts in Tema. Multivariate statistical analysis of the data was able to distinguish between participants from the two municipalities using a chemical fingerprint generated with only PBDD/Fs and PXDD/Fs. The results indicate that, over time, local sources of potential contamination (industrial areas and Agbogbloshie e-waste site) may impact populations that visit, work or live in close proximity to the site. We would recommend future studies to better establish the sources of dioxins and DLCs in Ghana, and potential trends over time. We also recommend that future biomonitoring studies on dioxins and DLCs include determination of PBDD/F and PXDD/Fs as this study indicates that their total contribution is significant.

615 **Strengths**

616 The main strengths of the present study are the congener-specific analysis of toxic
617 dioxin-like contaminants- PCDD/Fs, dlPCBs, PXDD/Fs and PBDD/Fs in maternal serum,
618 using targeted analysis. To the best of our knowledge this is the first study, in Ghana, to
619 examine maternal and foetal exposure to background concentrations of dioxins and DLCs.

620

621 As this study was focused on providing trace level targeted analysis of dioxins and DLCs, it
622 was impossible to complete a non-targeted analysis to determine the presence of other classes
623 of POPs. Future monitoring studies, could focus on non-targeted analysis to identify the varied
624 classes of toxic contaminants vulnerable Ghanaians are exposed to.

625

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634

635 **Financial Interest Declaration**

636 The authors declare no financial interests.

637

Ethical Approval

This study received ethical approval from the Ghana Health Service Ethics Review Committee (ref: GHS-ERC 04/08/16) on February 16th, 2017.

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