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Infant dietary exposure to dioxin-like polychlorinated biphenyls (dlPCBs), polybrominated and mixed halogenated dibenzo-p-dioxins and furans (PBDD/Fs and PXDD/Fs) in milk samples of lactating mothers in Accra, Ghana.

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Abstract

In this study, polybrominated and mixed halogenated dibenzo-p-dioxins and furans (PBDD/Fs and PXDD/Fs), and dioxin-like polychlorinated biphenyls (dlPCBs) were quantified in 24 human milk samples of first-time lactating mothers from Greater Accra region in Ghana. The aims of the study were to determine the concentrations and toxic equivalent concentrations of PBDD/F, PXDD/F and dlPCBs in human milk, and to estimate an infant's daily intake. The samples were analysed for 12 dioxin-like PCBs, 7 congeners of 2,3,7,8-polybrominated dibenzo-p-dioxins and furans (PBDD/Fs), and 7 congeners of 2,3,7,8-mixed halogenated dioxins and furans (PXDD/Fs, where X= Br/Cl). The mean concentrations in human milk ranged from 0.15-212.9 pg/g lipid for dlPCB congeners (mean TEQ: 1.67 pg WHO_{2005}-TEQ/g lipid). Lesser concentrations for 2,3,7,8-PXDD/Fs (and PBDD/Fs congeners) ranged between <0.01-1.67 pg/g lipid, with a total mean tentative TEQ of 0.56 pg WHO_{2005}-TEQ/g lipid. For an infant of average weight 7 kg, consuming an estimated volume of 600 mL human milk, the estimated average daily intake of dlPCBs in 21 human milk samples was 4.95 pg TEQ/kg bw/day; contributions from dlPCBs, PXDD/Fs and PBDD/Fs resulted in an average estimated daily intake of 6.56 pg TEQ/kg bw/day. The results obtained in this study, although lower than infant dietary intake estimates in human milk from industrialized countries, exceeded the recommended safety standards of 1 pg TEQ/kg bw/day and 1-4 pg TEQ/kg bw/day from the Agency for Toxic Substances and Disease Registry (ATSDR) and the World Health Organization (WHO), respectively.

1.0 Introduction

Dioxins and dioxin-like compounds (DLCs) are toxic classes of environmental contaminants that can be transferred from mother to foetus/baby via placenta/breastfeeding (Schecter et al., 2006; van den Berg et al., 2017; van Leeuwen et al., 2000a). Oral intake of foods by adults contribute over 90% of the total daily exposure to dioxins and DLCs. Similarly, human milk, although noted for its significance in protecting an infants' health, is a major source of toxic contaminants for a developing child (Djien Liem et al., 2000; Schecter et al., 1996; Schecter et al., 1998b; Victora et al., 2016). The daily intake of dioxins and DLCs by breastfed infants is linked to maternal body burdens. Due to bioaccumulation in lipid rich milk, an infant's exposure is approximately 2 orders of magnitude higher than that of an average adult (Djien Liem et al., 2000; Schecter et al., 1998b; Victora et al., 2016).

Dioxins and DLCs are produced from combustion and industrial processes of brominated and/or chlorinated organic compounds (Djien Liem et al., 2000; van den Berg et al., 2017; World Health Organization, 1996). The most toxic include 12 polychlorinated biphenyls (dioxin-like PCBs- dlPCBs), and 17 congeners of 2,3,7,8-polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) (Van den Berg et al., 2006). However, structurally related analogues of 2,3,7,8- mixed halogenated dioxins and furans (PXDD/Fs, X=Br/Cl), and polybrominated dibenzo-p-dioxins and furans (PBDD/Fs) exhibit similar toxicity profiles (Birnbaum et al., 2003; Olsman et al., 2007; Van den Berg et al., 2006).

Globally, the vast majority of biomonitoring studies of DLCs have assessed risks of infant/foetal exposure to intake of PCDD/Fs and dlPCBs in human milk/placental nutrients (Dewailly et al., 1991; Koopman-Esseboom et al., 1994; Schecter et al., 1998a; Schecter et al., 1998b; Schecter et al., 1990; Tanabe and Kunisue, 2007; van den Berg et al., 2017; Wittsiepe

et al., 2007). Relative to PCDD/Fs and dlPCBs, the risks of exposure of 2,3,7,8-PBDD/Fs and PXDD/Fs for breastfed infants are understudied. These classes have been found to contribute to the very limited literature on brominated and mixed halogenated dioxins, and significantly to the total Toxic Equivalents (TEQs) in biomonitoring studies of vulnerable populations (Bruce-Vanderpuije et al., 2019b; Jogsten et al., 2010; Ohta et al., 2004; Pratt et al., 2013). Additionally, in occupationally exposed individuals (firefighters), PBDD/F TEQ concentration in serum was approximately 20 times higher than for contributions from PCDD/Fs (Shaw et al., 2013). Since the majority of studies performed suggest a linkage to neuro-developmental and endocrine disrupting effects from exposure to dioxins and DLCs, investigation of human biological matrices will allow for communication of biomonitoring data to population groups most susceptible to exposure/high risks (Ames et al., 2018; Drover et al., 2019; Nakajima et al., 2017; Sethi et al., 2019).

In contrast to the numerous studies that have reported dioxins and DLCs in human milk in industrialized countries, most developing African countries have participated in only one global baseline survey conducted by the World Health Organization/United Nations Environment Programme (WHO/UNEP) (van den Berg et al., 2017). Exposure risks from DLCs in human milk have been found to be predominantly higher in industrialized countries compared to developing countries (Van den Berg et al., 2006; van den Berg et al., 2017; van Leeuwen et al., 2000a; World Health Organization, 1996). The majority of infant exposure studies have reported concentrations that exceed United States Environmental Protection Agency (USEPA) safe levels of 0.2 pg TEQ/g lipid [for PCDD/Fs + dlPCBs] in human milk (Djien Liem et al., 2000; Focant et al., 2002; Schecter et al., 1996; van den Berg et al., 2017; World Health Organization, 1996). Reported TEQ-concentrations of PCDD/Fs in human milk from developing countries showed a lower mean value of ~10 pg/g International (I)-TEQ/g

112 milk fat whilst industrialized countries had a range between 10-35 pg/g I-TEQ/g milk fat
113 (World Health Organization, 1998). Specifically, for Ghana, a developing country situated on
114 the southernmost part of West Africa, exclusive breastfeeding is encouraged for a minimum of
115 6 months. TEQ concentrations obtained from studies completed by the WHO/UNEP survey,
116 and by Adu-Kumi et al. (2010), were approximately 5 pg TEQ/g lipid and 6.1 pg TEQ/g lipid
117 in 50 pooled and 42 individual human milk samples (for PCDD/Fs + dlPCBs), respectively
118 (Adu-Kumi et al., 2010; van den Berg et al., 2017). It is important to note that these studies
119 only focused on dlPCBs and 2,3,7,8-PCDD/Fs. As brominated and mixed halogenated
120 dioxins/furans have similar mechanisms of action, their toxicity is possibly additive to that of
121 other DLCs. Thus, exclusion of 2,3,7,8-PBDD/Fs and PXDD/Fs may lead to underestimation
122 of the total TEQ and potential health risks.
123
124 A limited number of investigations on POPs in environmental matrices in Ghana have
125 been undertaken, and these have focused on chlorinated pollutants- organochlorine pesticides
126 (OCPs), polychlorinated biphenyls (mostly indicator PCBs) in environmental and food
127 matrices, and non-dlPCBs in human biomonitoring studies (Bruce-Vanderpuije et al., 2019a).
128 Little is known about exposure of vulnerable and occupationally-exposed populations to
129 dioxins and DLCs, especially with respect to brominated and mixed halogenated analogues.
130 Currently, there are a few studies that have focused on Ghanaian human biomonitoring; these
131 include studies on dioxins and DLCs in sera of occupationally exposed e-waste workers at
132 Agbogbloshie (Dai et al., 2020; Wittsiepe et al., 2015a), and background exposure
133 concentrations in sera of primiparous Ghanaians (Bruce-Vanderpuije et al., 2019b).
134 Additionally, low concentrations of PCB 118 (amongst other indicator PCBs) in human milk
135 samples from lactating mothers residing in Accra, Kumasi and Tamale in 2010 ranged between
136 1.9 and 3.0 ng/g lw (Asante et al., 2011). Data obtained from a baseline study for sera of

primiparous Ghanaians indicated a background exposure of foeti to dlPCBs (1.25 pg WHO-TEQ/g lw), PCDD/Fs (3.10 pg WHO-TEQ/g lw) and PXDD/Fs & PBDD/Fs (0.99 pg WHO-TEQ/g lw) in comparison to other studies reported globally. The significant contributions of brominated and mixed halogenated dioxins and furans (~ 20%) in sera of exposed populations to the total TEQs indicate possibilities of varied exposure sources including combustion, and dietary exposure.

A limited number of studies focusing on PXDD/Fs and PBDD/Fs have been published globally in human milk (Croes et al., 2013; Kotz et al., 2005; Ohta et al., 2004; Pratt et al., 2013; Tue et al., 2014; Wiberg et al., 1992). In a previous study on dioxins and DLCs in sera of primiparous Ghanaians, PXDD/Fs (and PBDD/Fs) contributed about 20% of the total TEQ (Bruce-Vanderpuije et al., 2019b). Based on the possibilities of transfer of dioxins and DLCs in human milk from an exposed mother to her baby, and the lack of quantified dioxins and DLC data in human biomonitoring studies in Ghana, the aims of this study were to determine the relative risk to infants from the consumption of human milk containing DLCs. This was achieved by the:

1) Determination of concentrations and TEQ concentrations of 12 dlPCBs, 7 analytes of 2,3,7,8-PBDD/Fs and PXDD/Fs in 24 Ghanaian human milk samples from first-time lactating mothers, with no known occupational or accidental exposure, and
2) Calculation of Ghanaian infant dietary intake from exclusive breastfeeding.

From the exposure risk calculations, the intake values of dioxin and DLCs in Ghanaian human milk samples obtained in this study were compared with recommended safety standard values and other studies reported globally, to determine possibilities of risk to breastfed infants.

2.0 Materials and Methods

2.1 Participant Recruitment

In 2017, 24 first-time lactating Ghanaians (primparous) who reside in industrialized areas of Accra were voluntarily recruited by research nurses from Ridge Regional hospital where they receive prenatal care. Participants completed written informed consent forms and exposure assessment questionnaires during regular prenatal visits, prior to birth and sample collection. Data on age, occupation, diet, cigarette smoking, alcohol intake and bodyweight were documented. Human milk samples (20 mL, n = 24) were collected in April, 2017, during the first two weeks after delivery, into 50 mL corning tubes and stored at -20 ^{o}C prior to extraction and analysis. The study was approved by the Ethics Review Committee of the Ghana Health Services, and conducted in accordance with ethical principles for medical research involving human subjects.

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). Octadecyl non-endcapped bonded silica C_{18} cartridges (10 g/75 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 dlPCBs, PBDD/Fs and PXDD/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 dlPCBs, PBDD/Fs 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 PXDD/Fs, PBDD/Fs and dlPCBs have been described in detail in a previously published paper on sera of primiparous Ghanaians (Bruce-Vanderpuije et al., 2019b). PBDD/F, dlPCB, and PXDD/F analytes analysed in this study are listed in Table S2. All standards were prepared in nonane, except for recovery spiking solutions which was prepared in methanol.

2.3 Sample Extraction

Sample extraction used in this study was based on previously validated analytical method (Focant and De Pauw, 2002) with minor modifications. The analytical method involved spiking 10 mL human milk sample with 5 µL of 2 pg/µL label recovery mix- 12 congeners of $^{13}C_{12}$-dlPCBs and 2,3,7,8-substituted congeners of $^{13}C_{12}$-PXDD/Fs and $^{13}C_{12}$-PBDD/F (3-B-2,7,8-CDF, 2,3-B-7,8-CDF, 2.3-B-7,8-CDD, 4-B-2,3,7,8-CDF, 1,3-B-2,7,8-CDF, BDD-2,3,7,8)- to determine extraction efficiency, matrix effects on recovery and enable quantitation by isotope dilution mass spectrometry. Extraction of lipophilic dioxins and DLCs was performed on human milk samples using C_{18} SPE (10 g/75 mL) with acetonitrile and water, after addition of sodium oxalate (20 mg/1 g milk) to disrupt the fat globules. Ten millilitres of acetonitrile and 10 mL water were added. C_{18} cartridges were conditioned gravimetrically using two cartridge volumes of acetonitrile and water prior to loading human milk mixture. Sample was eluted at a flow rate of 5 mL/min. Human milk tubes were rinsed with 2 x 10 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 2 x 10 mL hexane and collected in clear EPA vials, at a flow rate of 5 mL/min. All extracts were evaporated, and the extracted lipids were determined gravimetrically after solvent evaporation. Of the 24

participants that were sampled, 3 human milk samples were omitted because of technical difficulties during sample preparation.

2.4 Lipid removal Clean-up and Fractionation

Extracts were evaporated to 1 mL, and 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 acetonitrile. Eluate was evaporated under N_2 to 1 mL, and solvent was exchanged for hexane. A 5 g acidified silica column, connected to 2% ultra clean carbon mini column was activated with 20 mL hexane. The human milk extract was loaded, and cartridge was rinsed with 30 mL hexane. The silica cartridge was replaced with reusable glass column reservoir. The carbon column was inverted, and eluted with 30 mL toluene. Eluate was collected in 40 mL EPA vial, 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.

2.5 Instrumental Analysis

The instrumental method used for the analyses of the human milk samples has been previously reported in an earlier study developed for sera of pregnant Ghanaian women (Bruce-Vanderpuije et al., 2019b). Briefly, sample analyses were performed using a GC-APCI-MS/MS (Waters - Xevo TQ-XS). One microlitre of sample was injected in splitless mode on a DB5-MS column (60 m x 0.25 mm x 10 µm), using an oven program optimized for separation of dlPCBs, PBDD/Fs and PXDD/Fs. Instrumental parameters and operating conditions are summarized in Table S1. The mass spectrometer was operated in positive ion mode, using multiple reaction monitoring. Four transitions (2 quantifiers and 2 qualifiers for native and $^{13}C_{12}$ label components) were monitored for dlPCBs, PBDD/Fs and PXDD/Fs. The transitions,

collision energies and isotope ratios are summarized in Table S2. Samples were analysed using capillary gas chromatography with atmospheric pressure chemical ionization (APCI) and triple quadrupole tandem mass spectrometry (GC-APCI-MS/MS, Xevo TQ-XS) from Waters Corporation, Manchester, UK.

2.5.1 Quality assurance/ Quality Control

Analytes were quantified by isotope dilution using the ions specified in Table S2. For linearity, the response obtained for a native, relative to its corresponding ^{13}C labelled standard was linear for the range of calibration standards analyzed. Calculated coefficient of determination for dlPCBs and PXDD/Fs (and PBDD/Fs) were $R^2 \geq 0.998$ and $R^2 \geq 0.995$ respectively. The % RSDs obtained for dlPCBs and PXDD/Fs and PBDD/Fs) ranged between 1.2 and 13.2%; this is in agreement with the acceptable 15% value (Centre for Diseases Control and Prevention, 2006). Recoveries for isotopically labelled standards, spiked into human milk prior to extraction/clean-up, ranged between 50-80% (recoveries obtained fell within the acceptable EPA ranges). Where concentrations were below the LOD, ½ LODs were assigned and used in TEQ calculations. The instrument limit of quantitation was set at 5 fg/µL which represents the lowest detectable concentration in a calibration standard. The lipid adjusted human milk concentrations of dioxins and DLCs were reported as pg/g lipid. The Toxic Equivalent (TEQ) dlPCBs was calculated by multiplying the concentration (pg/g lipid) of each congener by its Toxic Equivalency Factor (TEF) value. For PXDD/Fs and PBDD/Fs, tentative TEQs were calculated using the assigned TEF values for polychlorinated dibenzo-p-dioxins/furans (Van den Berg et al., 2006).

2.5.2 Statistical Analyses

Data for dioxins and DLCs in human milk were statistically analysed using the Minitab 18 software package (Minitab, 2010). Descriptive statistics were performed on 21 human milk samples. Exploratory data analysis on histograms demonstrated that the levels of all congeners of dlPCBs, PXDD/Fs and PBDD/Fs follow a lognormal distribution, thus the geometric means were reported rather than the mean.

3.0 Results

3.1 Food consumption

Questionnaire responses on food consumption included major dietary intake of seafood, fish, meat and meat products, and dairy products. The majority of participants reported a similar consumption pattern. Seafood consumed included shrimp, 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.

3.2 Characteristics of lactating mothers

The subjects were lactating mothers aged between 21-32 years, at the time they gave birth; the mean age was 24 years. The average body mass index (BMI), calculated from the height and weight, was approximately 27.5 kg/m^2. The lipid concentrations measured in the human milk samples ranged between 3.2-4.2% (with a mean concentration of 3.7% ± 0.27: Table 3).

3.3 Congener specific dioxins and DLCs identified

A summary of results of the congener-specific concentrations of dlPCBs, 2,3,7,8-PXDD/Fs and 2,3,7,8-PBDD/Fs in human milk samples, and TEQ concentrations are shown in Tables 1, 2 and 3. Dioxin-like PCBs were detected in all 21 samples; all congeners detected were above the limit of detection (LOD). Figure 1 summarizes TEQ contributions of dlPCBs, PXDD/Fs and PBDD/Fs from each participant's human milk sample. An evaluation of the relative percentages of each congener to the total concentration showed the highest percentage mean contribution from PCB-156 (27%), and the highest percentage TEQ contribution from PCB-126. PCBs 81 and 169 contributed the least (3.6%) to the sum of average dlPCBs; PCBs

105, 156 and 167 contributed the most (22-26%) towards the overall sum of dlPCB congeners. Comparatively, in European countries, in addition to PCBs- 118, 167, and 105, PCB-156 was reported as one of the congeners with the highest concentration in human milk (Chovancová et al., 2011; Ulaszewska et al., 2011). These observations are in agreement with the concentrations detected in the current study.

3.4 Estimate of daily exposure of infants to TEQs from the consumption of human milk containing DLCs.

The concentrations of dioxins and DLCs in participants were within two orders of magnitude; concentrations were in order of dlPCBs >> PBDD/Fs > PXDD/Fs in human milk. The average total concentrations for 21 human milk samples ranged between 0.76 and 261 pg/g lw for all congeners studied (Tables 1-3). Percentage mean contributions from PXDD/Fs showed 2,3-B-78-CDD amounted to the lowest contribution of 1.36%; 1,3-B-2,7,8-CDF (30.9%) contributed the highest amount to the total mean PXDD/Fs. Percentage contributions of furan concentrations to the total concentrations were dominated by 1,2,3,4,6,7,8-BDF and 2,3,7,8-BDF. The mean TEQ concentration calculated for dlPCBs was 1.67 pg WHO_{2005}-TEQ/g lipid (Table 1); tentative TEQs calculated for 2,3,7,8-PBDD/Fs and 2,3,7,8-PXDD/Fs were 0.29 pg WHO_{2005}-TEQ/g lipid and 0.26 pg WHO_{2005}-TEQ/g lipid (Table 2), respectively. The mean TEQ for the sum of dlPCBs, 2,3,7,8-PXDD/Fs and 2,3,7,8-PBDD/Fs in 21 Ghanaian human milk samples was 2.23 pg TEQ/g lipid.

3.5 Assessment of estimated daily intake of TEQs by infants from consumption of human milk containing dlPCBs, PXDD/Fs and PBDD/Fs (2,3,7,8).

A risk assessment for breastfed infants was determined using the baseline data (for PXDD/Fs and PBDD/Fs) and dlPCBs presented in Table 3. Considering exclusive breastfeeding (for a minimum of 6 months) as the only exposure source to infants, an evaluation

of the daily intake and body burdens of dlPCBs, PXDD/Fs and PBDD/Fs was conducted based on the following assumptions:

1) An approximate/average volume of human milk consumed by an infant per day until its first year was estimated to be 600 mL milk per day, assuming an approximate human milk density of 1.03 g/mL (Focant et al., 2002; Schecter et al., 1998b).
2) An average body weight of an infant to be 7 kg (Focant et al., 2002).

To estimate the daily TEQ intake of dioxins and DLCs (pg TEQ/day) in human milk, the equations below were used.

Daily TEQ intake (pg TEQ/day) = TEQ concentrations measured in human milk (pg TEQ/g) x $\frac{Mass\ of\ breastmilk\ consumed\ (g)}{day}$ x Lipid (%) Equation 2

Daily TEQ intake (pg TEQ/day) per kg = $\frac{Daily\ TEQ\ intake\ (pg\frac{TEQ}{day})}{Estimated\ average\ body\ weight\ of\ infant}$ Equation 3

3.6 Discussions

Maternal diet impacts concentrations of dioxins and furans in human milk. Responses to dietary consumption on the questionnaire showed similarities in dietary pattern for all lactating mothers, for this study; thus, a correlation between age, food type, congener type could not be assessed. The prevalent food types (meat, fish, shellfish, dairy products sampled from industrialized areas) consumed have been reported to show PXDD/F contributions from fish (60%), shellfish (98%) and most edible terrestrial food products (Fernandes et al., 2014). Reporting PXDD/F concentrations in food consumed, their congener profiles, and correlations with specific food type by lactating mothers is beyond the scope of this study.

Table 4 summarizes the mean TEQ concentrations and infant estimated dietary intake in human milk from different countries reported globally on dlPCBs, PCDD/Fs and PXDD/Fs (Adu-Kumi et al., 2010; Deng et al., 2012; Focant et al., 2013; Focant et al., 2002; Harrison et al., 1998; Ohta et al., 2004; Papke, 1999; Pratt et al., 2013; Schecter et al., 1994; van den Berg et al., 2017; van Leeuwen et al., 2000b; Yang et al., 2002). Calculations on estimated dietary intake for nursing infants from industrialized countries such as Belgium, United States, United Kingdom, Germany, Sweden, China, France and Korea show that the estimated dietary intake of dioxins and DLCs in human milk ranged between 24-145 pg TEQ/kg bw/day, and was higher than the daily intake observed in Ghana. The estimated mean dietary intake of dlPCBs, PBDD/Fs and PXDD/Fs in 600 mL human milk (per day) for an infant of average weight, 7 kg, in this study is 6.5 pg TEQ/kg body weight (bw)/day; the range of intake varied from 2.4 to 29.2 pg TEQ/kg bw/day. The TEQ concentration estimated for infants in this study (6.5 pg TEQ/kg bw/day) does not include TEQ contributions from PCDD/Fs. Using the upper end WHO TDI safety standard assigned value of 4 pg TEQ/kg bw/day, the Agency for Toxic Substances and Disease Registry (ATSDR) reference dose (RfD) standard of 1 pg TEQ/kg bw/day, and the United States Environmental Protection Agency (0.7 pg TEQ/kg bw/day), 71% of infants' mean daily intake [dlPCBs + PXDD/Fs + PBDD/Fs] calculated in this study exceeded the recommended standard values. From the current study, a breakdown of contribution from dlPCBs to the daily estimated dietary intake indicated an approximate range between 1-28 pg TEQ/kg bw/day. Sixty-seven percent of infants were below the WHO estimated dietary intake of 4 pg TEQ/kg bw/day (upper end); all infants exceeded the recommended estimated daily intake of 1 pg TEQ/kg bw/day for dlPCBs. This indicates that although concentrations and TEQ concentrations detected from infant dietary intake in Ghana are much lower than that reported globally, the risks of

Ghanaian infants to toxic dioxins and DLCs from human milk intake are still high during periods of nursing.

The current concentrations, and TEQ concentrations of dlPCBs detected in this study from first time lactating mothers are lower than the approximate baseline dlPCB mean concentrations detected in 2008 in studies of Adu-Kumi et al. (2010): 3 pg TEQ/g lw for individual human milk samples, and the calculated value of 2 pg TEQ/g lw for pooled human milk in the WHO-UNEP global survey monitoring study in Ghana (Adu-Kumi et al., 2010; van den Berg et al., 2017). Additionally, the TEQ concentration estimated for infants in this study are for PBDD/Fs and PXDD/Fs; the overall TEQ concentration does not include TEQ contributions from PCDD/Fs, which appear to be the major contributor to the total TEQs in human milk in Ghana in studies of Adu-Kumi et al. (2010). A decrease in concentration of dlPCBs over a period of 9 years can be noted, although increases of dioxins and DLCs pollution have been reported in e-waste recycling areas in Accra (Tue et al., 2016; Wittsiepe et al., 2015b).

An assessment of tetra, penta and hexa PBDD/F and PXDD/F concentrations reported in literature showed that a number of studies did not find measurable amounts to quantify in human milk (Wiberg et al., 1992). Additionally, for studies which reported concentrations, relatively low contributions to the total TEQ were observed (Croes et al., 2013; Pratt et al., 2013). Typical concentrations detected (pg/g lipid) in pooled human milk samples in the above studies ranged between non-detect to 0.7 pg/g lipid for 2,3,7,8-PBDD/Fs in Flanders in studies of Croes et al. (2013). The mean concentrations of 2,3,7,8-PBDD/Fs and 2,3,7,8-PXDD/Fs reported in studies of Pratt et al. (2013) in pooled Irish human milk ranged from 0.05 to 1.36 pg/g lipid, and 0.02-0.54 pg/g lipid, respectively (Croes et al., 2013; Pratt et al.,

417 2013). The sum of mean concentrations of 2,3,7,8- PXDD/Fs and 2,3,7,8-PBDD/Fs identified
418 in the current study were 0.76 and 2.1 pg/g lw, respectively; these are in agreement with the
419 relatively low concentrations reported in literature. However, in another study on 2,3,7,8-
420 PBDD/Fs and 2,3,7,8-PXDD/Fs detected in Japanese human milk, high concentrations of
421 2,3,7,8-PBDD/Fs were reported with an average concentration of 269 pg/g lipid; mean
422 PXDD/Fs reported ranged between 0.68 to 12 pg/g lipid (Ohta et al., 2004).
423
424 Contributions from PCDD/Fs were not determined in this study; however, an estimated
425 dietary intake was calculated using previously reported TEQ values for PCDD/F in human
426 milk samples from Ghana from studies of Adu-Kumi et al. (2010) and the WHO global
427 survey result on Ghanaian infants. Thus, assuming a 3.5% lipid content, and a daily intake of
428 600 mL human milk/day, an estimated dietary intake of 9 pg TEQ/kg bw/day [PCDD/Fs] is
429 expected for human milk consumed by nursing infants in 2008. In an absence of known
430 accidental or occupational exposure, the major source of dioxins and DLCs, and their
431 respective TEQ concentrations in human milk of lactating mothers are likely to be attributed
432 to consumption of dairy products, meat and meat products, fish and seafood. This is further
433 corroborated by the relatively low concentrations when compared to other global values. The
434 results from this study were compared against concentrations detected in serum samples
435 obtained from primiparous Ghanaians (Bruce-Vanderpuije et al., 2019b). The lipid corrected
436 total concentration of dlPCBs in human milk samples (261 pg/g lipid) was greater than for
437 concentrations reported in serum (77.7 pg/g lipid). The calculated TEQ concentration of
438 dlPCBs is human milk was slightly higher (1.24 pg/TEQ/g lipid: sera and 1.67 pg/TEQ/g
439 lipid: human milk). The difference in total concentrations were attributed to higher
440 concentrations of the more chlorinated PCBs in milk samples: namely 189, 157, 167 and 105.
441 Lipid corrected total concentration of PXDD/F in human milk samples (0.75 pg/g lipid) and

sera were comparable (0.90 pg/g lipid); however, the calculated TEQ concentration of PXDD/F was greater in sera (0.26 pg/TEQ/g lipid: human milk and 0.50 pg/TEQ/g lipid: sera). This difference was due to higher concentrations of the highly potent 2,3-B 7,8-CDD detected in sera. Lipid corrected total concentrations of PBDD/F in human milk and sera samples (2.05 pg/g lipid: human milk, sera: 2.39 pg/g lipid) were comparable. However, the calculated TEQ concentration of PBDD/F was greater in sera (0.28 pg/TEQ/g lipid: human milk and 0.49 pg/TEQ/g lipid: sera). This difference was due to higher concentrations of the highly potent 2,3,7,8-BDD and 12,3,7,8-BDD detected in the sera. The data indicates the potential for preferential accumulation of certain DLCs in human milk when compared to sera. This is interesting considering that previous studies on PCB profiles in 15 different tissue types from the same organisms have been found to be largely consistent (Megson et al., 2018). However, it should be noted that samples from the milk and sera were obtained from different participants at different times; therefore, further research is required to verify any differences observed between the two matrices.

4.0 Conclusion

This first cumulative study on brominated and mixed halogenated dioxins and furans highlights Ghanaian infants to be at risk from dietary intake of dlPCBs, PBDD/Fs and PXDD/Fs from human milk; an average daily intake of DLCs in excess of the WHO recommended safety was estimated. The sum of average concentrations observed in 21 Ghanaian human milk samples were 261 pg/g lipid (dlPCBs), 2.05 pg/g lipid (PBDD/Fs), and 0.76 pg/g lipid (PXDD/Fs). The mean TEQ concentration was 2.23 pg TEQ/g, with contributions of 75.6% from dlPCBs, 13.3% from PBDD/Fs and 11.1% from PXDD/Fs. The calculated mean infant daily intake for dlPCBs was 4.95 pg TEQ/kg bw/day; a total average daily intake of 18.30 pg TEQ/kg bw/day was estimated when also considering existing PCDD/F and dlPCB data from previous Ghanaian studies. The major contributors to the background concentrations of dioxins and DLCs and their respective TEQ concentrations in human milk of Ghanaian lactating mothers can likely be attributed to consumption of dairy products, meat and meat products, fish and seafood. The exposure assessment performed indicates that all infants consuming human milk exceeded the recommended standard intake of 1 pg TEQ/kg bw/day (as set by the ATSDR & WHO). Provided an individual continually consumes dioxin and DLC-contaminated food from infancy to adulthood, the amount of daily intake of dioxins and DLCs would highly exceed the safety standards assigned by the ATSDR, the USEPA and the WHO. Given that a foetus/infant's developmental growth is affected by nutrients consumed transplacentally and via human milk, we suggest strategies by Ghanaian governing bodies be directed towards minimizing/reducing PCB and dioxin intake through the food chain. Within this work consideration should also be given towards brominated and mixed halogenated DLCs as well as the more traditional chlorinated analogues.

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Financial Interest Declaration

The authors declare no financial interests.

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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