



Characterization and quantification of endocrine disruptors in female menstrual blood samples

Benjamin Ason^{a,b,*}, Frederick Ato Armah^c, David Kofi Essumang^a

^a Department of Chemistry, School of Physical Science, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana

^b Soil Research Institute, Council for Scientific and Industrial Research, Accra, Ghana

^c Department of Environmental Science, School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana

ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:

Menstrual
Endocrine disrupting chemical
Blood
Ghana

ABSTRACT

Selected endocrine disrupting chemicals (EDCs) were measured in adult female menstrual blood for the first time in Ghana, Africa, taking into account the importance of non-invasive means of matrices sampling in vulnerable groups, such as pregnant women, the elderly or chronically ill people. The menstrual blood samples of twenty (20) female adults between the ages of 25–45 years were sampled. The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method was applied for the extraction and clean up, while gas chromatography-mass spectrometry (GC-MS) was used to measure the selected EDCs in adult female menstrual blood, taking into account the composition of menstrual discharge. Diethyl phthalate (DEP), Dibutyl phthalate (DBP) and Bis (2-ethylhexyl) phthalate (DEHP) were detected in all samples, whereas bisphenol A (BPA) was found in 13 participants. Dimethyl phthalate (DMP) was detected in 7 participants, Di-n-octyl phthalate (DNOP) was detected in 3 participants, Bis (2-ethylhexyl) adipate (DEHA) and pyrimidine were detected in 2 participants, while benzyl butyl phthalate (BBP) was detected in only 1 participant. The maximum concentration of DEP measured was 115.6 $\mu\text{g}\cdot\text{L}^{-1}$ and the minimum was 439 $\mu\text{g}\cdot\text{L}^{-1}$. DEHP was the next most abundant phthalate with a maximum measured concentration of 982 $\mu\text{g}\cdot\text{L}^{-1}$ and minimum of 95 $\mu\text{g}\cdot\text{L}^{-1}$. The presence of parent phthalates (rather than metabolites) in menstrual blood of all participants studied suggests that bioaccumulation of selected phthalate compounds such as DEHP, DEP and DBP may be occurring with appreciable human toxicity though the carcinogenic exposure risks of DEHP via various routes were much lower than 1×10^{-6} considered to be very low.

1. Introduction

Endocrine disrupting chemicals (EDCs) consist of many natural and synthetic organic compounds that are known to interrupt normal function of the endocrine system in various ways. This class of chemicals is usually found in plastic products, most household products, as well as the environment [5,23]. Human exposure pathways to EDCs are mainly through the intake of food, water and dust, inhalation of gases and particles and dermal contact [27]. EDCs have been found in surface water, sediment, groundwater, and even drinking water in many countries [7,14]. They can interfere with the production of hormones needed for the normal functioning of the human body either through over or underproduction of hormones or by disrupting hormone production [18,23]. Since these hormones are required in small quantities and at specific moments to regulate the body's growth.

EDC exposure is associated with changes in reproductive function and high occurrence of cancers [8]. Among females, EDCs may impair reproductive function across the life cycle, such as inducing early puberty or increasing rates of miscarriage and infertility [11,45]. EDC exposure is also associated with increased risk of reproductive cancers especially breast, ovarian, and endometrial cancers, neurodevelopmental delays and abnormal growth patterns in children, and changes in immunity [10,15,23]. The impact of EDCs on female health is critical since these compounds can stimulate estrogenic responses at very low concentrations that could affect pregnant mothers and their unborn babies [15,37]. EDCs can also be transferred from a pregnant woman to the growing fetus through the placenta and to the baby through breast milk. Adult exposure to EDCs may have different consequences than exposure to a developing fetus or infant [11]. Studies show that exposures to EDCs and concomitant health effects may not

* Corresponding author at: Department of Chemistry, School of Physical Science, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana.

E-mail addresses: soilresearchben@gmail.com (B. Ason), farmah@ucc.edu.gh (F.A. Armah), dessumang@ucc.edu.gh (D.K. Essumang).

<https://doi.org/10.1016/j.toxrep.2022.10.007>

Received 1 August 2021; Received in revised form 29 September 2021; Accepted 11 October 2021

Available online 10 October 2022

2214-7500/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

manifest until later in life [15]. The exposure of women is heightened with the increased use of personal care products and chemical products that may contain EDCs.

In epidemiologic research, EDCs are commonly measured in human biological matrices such as blood, urine or breast milk. Other matrices, such as saliva, amniotic fluid or hair have also been used in laboratory analysis of exposure to EDCs. However, little is known about the potential use of menstrual blood in exposure analysis. Careful choice of a particular matrix is important as toxicokinetics, stability, specificity and reliability should be considered. Research based on the use of menstrual blood is scarce probably due to the difficulty in obtaining it, especially in Africa where a lot of myth is attributed to it. Nonetheless, menstrual blood, like other matrices, could be used to obtain vital information on exposure and disease.

Humans may come into contact with environmental contaminants through several routes [27]. Monitoring of contaminants is essential for the determination of their effects on individuals. Though the use of pesticides and other chemicals cannot be avoided entirely, it is imperative to ensure that adverse health effects of products that contain EDCs are understood and monitored to curb the upsurge in use. Again in monitoring, there are various considerations for the selection of a suitable matrix for human biomonitoring of contaminants. These include gender, age, frequency of monitoring and the type of contaminant. Depending on the purpose, invasive or non-invasive methods of matrix sampling could be employed. Blood has been used widely due to its interaction with all tissues and organs [21]. However, blood sampling is an invasive procedure accompanied with practical constraints, particularly for children or other susceptible populations [30,36]. In addition, frequent biomonitoring is necessary for the evaluation of risk management options and effectiveness of environment and health policies [4]. For chemicals with short half-lives such as volatile organic compounds or agricultural pesticides, repeated sampling of exposure provides more insight into the true nature of these chemicals and their toxicological consequences. In these situations, non-invasive means of matrix collection is preferable, particularly in vulnerable groups, such as pregnant women, elderly or chronically ill people. Since non-invasively collected matrices need less specialized personnel for sampling, costs associated with large sampling designs may be significantly reduced compared to invasive collection of matrices [35].

Considering the advantages, there is a strong case for non-invasive collection of matrices for human biomonitoring as an ethically appropriate, cost-efficient and toxicologically relevant alternative for many of the biomarkers currently measured in invasive matrices. However, the use of menstrual discharge sampled non-invasively as a biomonitoring matrix has been minimal, likely due to the myth surrounding it in Africa. In other jurisdictions however, menstrual matrices have been used in a few instances for human biomonitoring [20,33]. Menstrual discharge is composed of three distinct body fluids including blood, vaginal secretions, and the endometrial cells of the uterine wall [19,44]. It may therefore be suitable for the monitoring of females within child bearing age and offers an advantage of early detection of contaminants before conception. In Ghana, the use of plastics in daily life as food wrappers and for storage is pervasive. Increased use of plastic products, pesticides, personal care products, pharmaceuticals and industrial chemicals has occurred during the past decade. These products contain phthalates and bisphenol A that are ubiquitous in the environment due to their widespread application. The high molecular weight phthalates (DEHP, DiNP, DiDP), are used primarily in PVC polymers, plastics, food packaging, food processing materials, vinyl toys, vinyl floor coverings, and building products. The low molecular weight phthalates (DMP, DEP, and DBP), are often used in non-PVC applications, such as personal care products, paints, adhesives, and enteric-coated tablets [43]. According to UNEP [39], in Ghana, over one million tons of plastic waste is generated annually and contributes 0.29 % of global plastic waste generated. Approximately, 81 % of plastic waste generated are mismanaged which end up in the environment [39]. Biomonitoring is scarce and the early

detection of harmful impacts of environmental contaminants is critical in the protection of neonates. Few studies in Africa have assessed levels of EDCs in biota particularly using menstrual blood as a non-invasive sampling approach. It is unclear whether adult female menstrual can be used as an indicator of EDC bioaccumulation or toxicity. The aim of the study was to measure selected EDCs in adult female menstrual blood while taking into account the composition of menstrual discharge as non-invasive method of sampling.

2. Materials and methods

2.1. Collection and storage of blood samples

Menstrual blood samples were collected by volunteers for three consecutive days from the second day of flow within the monthly cycle. Twenty (20) females from Accra and Cape Coast between the ages of 25–45 years participated in the study. Participants refrained from sex during sample collection. Since there is no standard protocol for the collection of menstrual blood, samples were collected on aluminum foil and transferred into a vacutainer tube containing anticoagulant. The collected blood samples were stored in sealed sterile containers at $-10\text{ }^{\circ}\text{C} (\pm 2\text{ }^{\circ}\text{C})$ until extraction. All subjects included in the study gave their consent to participate voluntarily. Ethical approval (GHS-ERC004/09/19) was granted by the Ghana Health Service Ethical Review Board.

2.2. Extraction and clean up

The modified method described by [41] was used for extraction and clean up. About 0.5 ml of blood serum was diluted with 1.5 ml of distilled water. Samples were placed in a 5 ml centrifuge tube containing 6 g of magnesium sulfate and 1.5 g sodium chloride with 1 ml of 1 % acetic acid in acetonitrile (v/v). The mixture was vigorously vortexed for 30 s, shaken up and down for 5 min, vortexed again for 30 s and centrifuged at 4400 rpm for 15 min at $4\text{ }^{\circ}\text{C}$. For sample clean up, 600 μl supernatant of each sample was transferred into a 1.5 ml tube containing the solid phase extraction sorbent (25 mg of primary-secondary amine, 25 mg of end-capped octadecylsilane (C18) and 150 mg of magnesium sulfate). The tube was mixed by hand (up and down) for 10 mins, vortexed for 30 s and centrifuged at 4400 rpm for 10 min at $4\text{ }^{\circ}\text{C}$. Supernatant were collected and filtered through 0.22PTEF filter into vials. About 1 μl of each sample was injected directly into GC-MS/MS system for analysis.

2.3. Analysis of selected EDCs

The blood samples were analyzed for selected EDCs with a GC-MS QP 2020 Shimadzu equipped with an RTS-5MS trace analysis column (30 m \times 0.25 mm \times 0.25 μm). Helium gas of high purity (99.99%) was used as the carrier gas at a flow rate was 1 ml min^{-1} at a pressure of 83.1 kp. Sample volume of 1 μl was injected in pulsed splitless mode with an inlet temperature of $265\text{ }^{\circ}\text{C}$.

To quantify phthalates, the column temperature was programmed as follows: initial oven temperature was set at $70\text{ }^{\circ}\text{C}$ for 2 min, then increased to $200\text{ }^{\circ}\text{C}$ at $15\text{ }^{\circ}\text{C}/\text{min}$. It was further ramped up at $6\text{ }^{\circ}\text{C}/\text{min}$ to $310\text{ }^{\circ}\text{C}$ and held for 1 min. The interface and ion source temperatures were set at $265\text{ }^{\circ}\text{C}$ and $220\text{ }^{\circ}\text{C}$ respectively. For bisphenol A, the column temperature program was as follows: $50\text{ }^{\circ}\text{C}$ for 1 min, ramped up at $15\text{ }^{\circ}\text{C}/\text{min}$ to $180\text{ }^{\circ}\text{C}$, further ramped up at $8\text{ }^{\circ}\text{C}/\text{min}$ to $300\text{ }^{\circ}\text{C}$ and held for 1 min. The interface and ion source temperatures were set to $270\text{ }^{\circ}\text{C}$ and $220\text{ }^{\circ}\text{C}$, respectively. Quantifications were all carried out in the selected ion monitoring mode (SIM), selecting two characteristic fragments ions for each.

2.4. Quality control

All glassware used was thoroughly washed with hexane and

acetonitrile, then heated at 140 °C for 1 h to ensure that contamination of glassware was reduced. The EDC extraction solvent and matrix adsorbent were studied using blank samples spiked with standards. The blank values of the analytical procedure were determined by extracting the spiked sample by the same method as the real blood sample with recovery of spiked sample in the range of 86–116%. The estimation of the limit of detection (LOD) for the selected EDCs in the blood samples was conducted based on U.S. EPA guidelines [40] with a confidence level of 95 %. An LOD of $3 \times$ (detection peak/blank peak + standard deviation) was observed. Instrumental detection limits were calculated by a signal-to-noise ratio of 3 times the sample concentration and ranged from 100 to 310 $\mu\text{g L}^{-1}$. Method detection limits of the selected EDCs including DMP, DEP, BBP, DEHP, DBP, DnOP, DEHA and BPA were 5, 7, 10, 5, 15, 10, 7 and 40 $\mu\text{g L}^{-1}$, respectively. Surrogate standards and internal standards concentrations of 20 μl of 5 ppm and 50 μl of 20 ppm in 1 ml, respectively, were added to all the samples to monitor the matrix effects, calibration and quantification. A calibration curve was made by serial dilutions of calibration standards with at least five concentrations for each selected EDC compound monitored. Linear regression with coefficient of (R^2) > 0.99 was accepted.

2.5. Human exposure and health risk assessment

The cancer risks (CR) of the endocrine disrupting chemicals in the menstrual blood samples were estimated according to the US EPA recommendation. The formulas with modification from the US EPA, [38] which have been widely employed in previous studies ([6]; [25]; [42,47]) were used to assess the average daily dosage (ADD, $\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) and cancer risk of the selected EDCs via different exposure pathways, namely ingestion (ADD_{ing}), dermal absorption (ADD_{der}) and inhalation (ADD_{inh}) as Eqs. (1)–(4).

$$ADD_{ing} = \frac{C_b \times I_g \times EF \times ED}{BW \times AT} \times CF \quad (1)$$

$$ADD_{der} = \frac{C_b \times SA \times AF \times ABS \times EF \times ED}{BW \times AT} \times CF \quad (2)$$

$$ADD_{inh} = \frac{C_b \times I_h \times EF \times ED}{PEF \times AT \times BW} \quad (3)$$

$$CR = \sum (ADD_j \times CFS) \quad (4)$$

Where C_b is the individual concentration of selected EDCs measured in blood ($\mu\text{g}\cdot\text{L}^{-1}$); I is the daily intake rate; (I_g) is the ingestion rate ($\text{mg}\cdot\text{d}^{-1}$); (I_h) is the inhalation rate ($\text{m}^3\cdot\text{d}^{-1}$). SA is the dermal exposure area (cm^2). ABS is the dermal adsorption fraction. AF is the dermal adherence factor ($\text{mg}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$). BW is the body weight (kg). AT is the averaging time (Days): for non-cancer risks, $AT = ED \times 365$; for carcinogens risks, $AT = \text{average lifetime} \times 365$. ED is the exposure duration. EF is the exposure frequency ($\text{days}\cdot\text{year}^{-1}$). CF is the conversion factor ($1.0 \times 10^{-6} \text{ kg}\cdot\text{mg}^{-1}$). PEF is the particle emission factor ($1.36 \times 10^9 \text{ m}^3\cdot\text{kg}^{-1}$). CSF represents the cancer slope factor. The carcinogenic risk of selected EDC was determined from Eq. (4). The estimated carcinogenic risks may be considered very low if the value of risk is less than 1×10^{-6} , low in the range of 1×10^{-6} to 1×10^{-4} , moderate from 1×10^{-4} to 1×10^{-3} , high from 1×10^{-3} to 1×10^{-1} and very high if the value is greater than 1×10^{-1} [29]. (Table 1).

3. Results

Assessment of organic contaminants in the menstrual blood samples of 20 female adults between the ages of 25–45 indicated the presence of both phthalates and bisphenol A; phthalates were more abundant across samples. Three contaminants: diethyl phthalate, dibutyl phthalate, and bis (2- ethyl hexyl) phthalate were detected in samples of all participants. BPA was present in blood samples of thirteen participants while

Table 1
Parameters for human health risk assessment.

Parameters	Reference value	Reference
I_g	100 (adults), 200 (children)	Kamunda et al.,[22,26]
I_h	20 (adults), 10 (children)	Kamunda et al.,[22,26]
EF	350	Kamunda et al.,[22,26]
ED	30	Kamunda et al.,[22]
BW	70 (adults), 15 (children)	Kamunda et al.,[22,26]
AT	70 _ 365 (carcinogenic), ED X 365 (non -carcinogenic)	Kamunda et al.,[22,26]
CF	1×10^{-6}	Kamunda et al.,[22,26]
AF	0.07 (adults),	Kamunda et al.,[22,26]
SA	5700 (adults),	(Y. Li et al.,[26])
ABS	0.1	Kamunda et al.,[22,26]
PEF	1.36×10^9	Kamunda et al.,[22,26]
CSFo (BBP)	0.00019	(Y. Li et al.,[26])
CSFo (DEHP)	0.014	(Y. Li et al.,[26])

DMP was detected in 7 participants. Table 2 indicates that only one participant had detectable levels of benzyl butyl phthalate, two participants had detectable levels of bis (2- ethyl hexyl) adipate and pyrimidine, and three participants had detectable levels of di-n- octyl phthalate and progesterone.

Three participants had detectable levels of six of the various contaminants, six participants had five contaminants, ten participants had four contaminants, and one of the participants had three contaminants as shown in Fig. 1.

Higher concentrations of DMP, DEP, DEHP, DBP and BPA were present in menstrual blood compared with BBP, DEHA, and DNOP that had negligible concentrations as indicated in Fig. 2.

Among the phthalates, DEP had the highest concentration followed by DEHP, DBP and then DMP. DEP, DEHP, and DBP were present in higher concentrations than BPA.

We observed that pyrimidine and DEHP showed much variability with a standard deviation of 0.532 and 0.288, respectively (Table 3). DEP and progesterone showed similar deviation from the mean, while the other contaminants had standard deviations of less than 0.1. DEP had the highest mean concentration of 824.8 $\mu\text{g}\cdot\text{L}^{-1}$, whereas DNOP had the lowest mean concentration of 0.65 $\mu\text{g}\cdot\text{L}^{-1}$. The maximum concentration of DEP recorded from one of the subjects was 115.6 $\mu\text{g}\cdot\text{L}^{-1}$ and the minimum was 439 $\mu\text{g}\cdot\text{L}^{-1}$. DEHP, the next most abundant phthalate had a maximum recorded concentration of 982 $\mu\text{g}\cdot\text{L}^{-1}$ and minimum recorded concentration of 95 $\mu\text{g}\cdot\text{L}^{-1}$.

3.1. Human exposure and health risk assessment

This study evaluated the CR of DEHP using a cancer slope factor of 0.014 via ingestion, inhalation and dermal contact based on the concentrations of the selected EDCs of the individual menstrual blood sample. Reference was made to the acceptable risk level recommended by the US EPA (1×10^{-6}) when estimating the lifetime excess CR of phthalate esters. The assessment of cancer risks via all the routes showed that none of the routes exceeded the recommended allowable level. The

Table 2
Percentage of individuals with detected contaminants in menstrual blood sample.

Contaminant	Percentage (n = 20)
Bisphenol A	65
Dimethyl phthalate	35
Diethyl phthalate	100
Dibutyl Phthalate	100
Benzyl Butyl Phthalate	5
Bis (2- ethyl hexyl) adipate	10
Bis (2- ethyl hexyl) phthalate	100
Di-n- octyl Phthalate	15
Progesterone	15
Pyrimidine	10

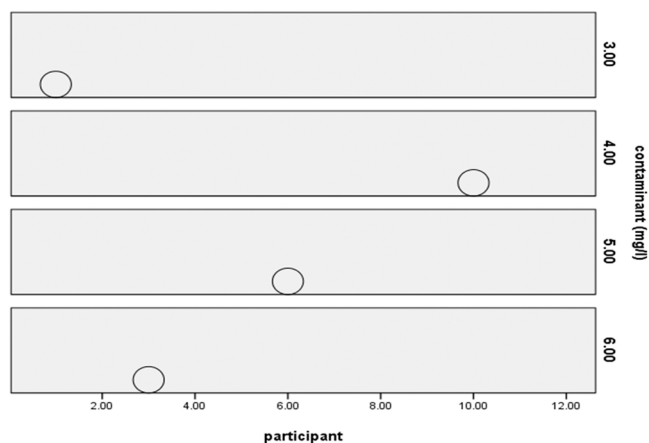


Fig. 1. Participants with number of distinct contaminants in menstrual blood sample.

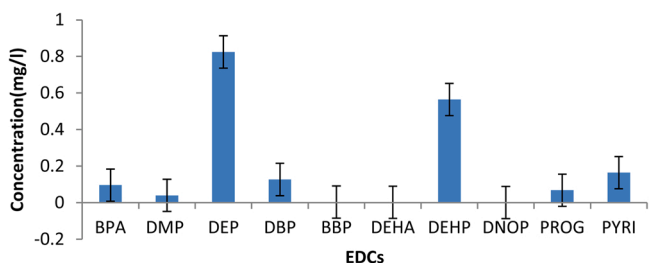


Fig. 2. Mean level of selected EDCs in menstrual blood sample of participants.

Table 3 Descriptive statistics of endocrine disrupting chemicals and progesterone measured in menstrual blood samples of 20 participants.

Contaminant ($\mu\text{g.L}^{-1}$)	Minimum	Maximum	Mean	Std. Deviation
BPA	.000	208	96	.074
DMP	.000	187	40	.067
DEP	439	1156	825	.173
DBP	.081	244	127	.035
BBP	.000	60	3	.013
DEHA	.000	47	4	.010
DEHP	95	982	565	.288
DNOP	.000	7	1	.002
PROG	.000	555	68	.170
PYRI	.000	2149	164	.532

carcinogenic exposure risks of DEHP via various routes were much lower than 1×10^{-6} . The results of the risk assessment are summarized in Table 4.

4. Discussion

Human exposure pathways to EDCs vary and obtaining information pertaining to exposure effects and diseases is mainly done by analyzing compounds in human biological matrices. In total, ten different compounds were detected in menstrual blood samples. Studies conducted on BPA in human biological matrices in Africa mostly measured urinary BPA [32]. The maximum concentration of BPA found in menstrual blood in this study was similar to the maximum concentrations found in urine samples reported by [1]. However, Gounden et al. [16] conducted a study on maternal and cord blood in which BPA concentrations lower than 0.208 ng/ml were detected. In neonates and infants, exposure to EDCs may occur through the placenta or via breast-feeding [28]. Lower blood BPA levels than those in this study were found in children and

Table 4 Health risk assessment of contaminant measured in menstrual blood sample of participants.

Sample ID	Ingestion	DEHP		
		Dermal	Inhalation	Total
1	2.532E-09	1.010E-10	3.723E-13	2.633E-09
2	5.786E-09	2.309E-10	8.509E-13	6.018E-09
3	1.159E-09	4.624E-11	1.704E-13	1.205E-09
4	8.005E-09	3.194E-10	1.177E-12	8.326E-09
5	3.929E-09	1.568E-10	5.778E-13	4.086E-09
6	1.093E-09	4.362E-11	1.608E-13	1.137E-09
7	6.542E-09	2.610E-10	9.621E-13	6.804E-09
8	1.068E-09	4.263E-11	1.571E-13	1.111E-09
9	3.616E-09	1.443E-10	5.318E-13	3.761E-09
10	6.649E-09	2.653E-10	9.778E-13	6.916E-09
11	5.959E-09	2.378E-10	8.763E-13	6.198E-09
12	3.460E-09	1.381E-10	5.089E-13	3.599E-09
13	6.625E-09	2.643E-10	9.742E-13	6.890E-09
14	3.616E-09	1.443E-10	5.318E-13	3.761E-09
15	5.926E-09	2.364E-10	8.715E-13	6.163E-09
16	5.326E-09	2.125E-10	7.832E-13	5.539E-09
17	7.808E-10	3.115E-11	1.148E-13	8.121E-10
18	6.625E-09	2.643E-10	9.742E-13	6.890E-09
19	8.071E-09	3.220E-10	1.187E-12	8.394E-09
20	6.049E-09	2.414E-10	8.896E-13	6.292E-09

pregnant women in China [46]. The identified concentrations could be attributed to the cumulative effect of three distinct body fluids that consist of blood, vaginal secretions and the endometrial cells of the uterine wall. It could also be attributed to the widespread use of plastics for both domestic and commercial purposes with over one million tons of plastic waste is generated annually which contributes 0.29 % of global plastic waste generated [39], considering the increased desire for the consumption of processed and packaged foods. The main sources of BPA and phthalates in blood are dietary sources such as food and drinks packaged in cans and polycarbonate bottles, as well as paper and plastic films [9]. These chemicals could leach from polycarbonate plastics and plastic films used for food packaging and storage containers [2].

The phthalate esters of DEP, DBP and DEHP identified were the most abundant compounds measured. A study carried out by Wang et al. [43] reported corresponding abundant compounds of phthalate metabolites of urine samples in a review of phthalate exposures.

Human exposure to phthalates is based mainly on the measurement of urinary monoester metabolites, although several secondary and oxidative metabolites have been reported to occur in human specimens [34]. Studies on EDCs in blood are few and even more scarce are studies on EDCs in menstrual blood within the sub-Saharan African context. The identification of phthalate esters (instead of metabolites) in menstrual blood among all participants studied suggests that bioaccumulation of selected phthalate compounds such as DEHP, DEP and DBP may be occurring with appreciable human toxicity. A similar observation of possible phthalate retention and bioaccumulation was made in a clinical study of phthalate elimination in blood, urine and sweat [12,13].

Progesterone is an essential hormone that affects reproductive health. The low detectable levels of progesterone among participants could indicate health effect of EDCs. Guo et al. [17]; Li et al. [24] indicated that there is ample evidence to suggest that endocrine disruptors may affect progesterone production in females.

Risk assessment is one of the approaches for investigating the potential risks of contaminant exposure on human health and providing important information. In this study the carcinogenic exposure risks of DEHP via various routes were much lower than 1×10^{-6} , risk limit suggesting that there is insignificant cancer risk for DEHP.

5. Conclusion

The study set out to measure selected EDCs in adult female menstrual blood as non-invasive method of sampling. Menstrual blood is an easily

obtainable body fluid and collected through non-invasive means. Bisphenol A, Dimethyl phthalate, Diethyl phthalate, Dibutyl Phthalate, Benzyl Butyl Phthalate, Bis (2- ethyl hexyl) adipate, Bis (2- ethyl hexyl) phthalate, Di-n- octyl Phthalate, and Pyrimidine were detected in female menstrual blood. Continuous human exposure to organic compounds that are endocrine disrupting in nature is an important issue that requires frequent monitoring. Hence, the use of menstrual discharge sampled non-invasively provides an alternative biomonitoring matrix critical to the protection of vulnerable groups, such as chronically ill people. The low detectable levels of progesterone among participants could indicate health effect of EDCs. The data obtained demonstrate that many proposed markers of women's health are present in the menstrual blood, which could subsequently be used to determine exposure to contaminants. Early detection of the harmful impact of environmental contaminants is critical to human protection, especially among neonates. The detection of phthalates in the menstrual blood of all study participants suggests that bioaccumulation of phthalate may be occurring with appreciable human toxicity.

CRedit authorship contribution statement

Benjamin Ason: Conceptualization, Methodology, Original draft preparation, Formal Analysis, Writing **Frederick Ato Armah:** Software, Data curation, Formal Analysis Reviewing and Editing **David Kofi Essumang:** Supervision, Reviewing and Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

- [1] H. Abo El-Atta, A. El-Mansoury, A. El-Hawary, M. Abdel-Naby, M. Helmy, Bisphenol-A and risk of obesity among a sample of Egyptian children: role of adiponectin as biomarker of exposure, *Mansoura J. Forensic Med. Clin. Toxicol.* 26 (1) (2018) 39–52, <https://doi.org/10.21608/mjfmct.2018.46570>.
- [2] G. Andaluri, M. Manickavachagam, R. Suri, Plastic toys as a source of exposure to bisphenol-A and phthalates at childcare facilities, *Environ. Monit. Assess.* 190 (2) (2018), <https://doi.org/10.1007/s10661-017-6438-9>.
- [4] S.M. Arnold, J. Angerer, P.J. Boogaard, M.F. Hughes, R.B. O'Lone, S.H. Robison, A. Robert Schnatter, The use of biomonitoring data in exposure and human health risk assessment: benzene case study, *Crit. Rev. Toxicol.* Vol. 43 (2013) 119–153, <https://doi.org/10.3109/10408444.2012.756455>.
- [5] S. Basak, M.K. Das, A.K. Duttaroy, Plastics derived endocrine-disrupting compounds and their effects on early development, *Birth Defects Res.* 112 (17) (2020) 1308–1325, <https://doi.org/10.1002/bdr2.1741>.
- [6] B. Başaran, G.N. Soyulu, M. Yılmaz Civan, Concentration of phthalate esters in indoor and outdoor dust in Kocaeli, Turkey: implications for human exposure and risk, *Environ. Sci. Pollut. Res.* 27 (2) (2020) 1808–1824, <https://doi.org/10.1007/s11356-019-06815-2>.
- [7] W. Brueller, N. Inreiter, T. Boegli, M. Rubasch, S. Saner, F. Humer, F. Allerberger, Occurrence of chemicals with known or suspected endocrine disrupting activity in drinking water, groundwater and surface water, Austria 2017/2018, *Bodenkultur* 69 (3) (2018) 155–173, <https://doi.org/10.2478/boku-2018-0014>.
- [8] E. Buoso, M. Masi, M. Racchi, E. Corsini, Endocrine-disrupting chemicals' (Edcs) effects on tumour microenvironment and cancer progression: emerging contribution of rack1, *Int. J. Mol. Sci.* 21 (2020) 1–27, <https://doi.org/10.3390/ijms21239229>.
- [9] J. Corrales, L.A. Kristofco, W. Baylor Steele, B.S. Yates, C.S. Breed, E. Spencer Williams, B.W. Brooks, Global assessment of bisphenol a in the environment: review and analysis of its occurrence and bioaccumulation, *Dose-Response* 13 (3) (2015), <https://doi.org/10.1177/1559325815598308>.
- [10] D.A. Crain, S.J. Janssen, T.M. Edwards, J. Heindel, S.M. Ho, P. Hunt, L. J. Guillette, Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing, *Fertil. Steril.* 90 (4) (2008) 911–940, <https://doi.org/10.1016/j.fertnstert.2008.08.067>.
- [11] E. Diamanti-Kandarakis, J.P. Bourguignon, L.C. Giudice, R. Hauser, G.S. Prins, A. M. Soto, A.C. Gore, Endocrine-disrupting chemicals: an endocrine Society scientific statement, *Endocr. Rev.* 30 (4) (2009) 293–342. (https://www.mendeley.com/reference-management/web-importer/#id_2).
- [12] S.J. Geniis, S. Beesoon, R.A. Lobo, D. Birkholz, Human elimination of phthalate compounds: blood, urine, and sweat (BUS) study, *Sci. World J.* (2012) 2012, <https://doi.org/10.1100/2012/615068>.
- [13] A. Giuliani, M. Zuccarini, A. Cichelli, H. Khan, M. Reale, Critical review on the presence of phthalates in food and evidence of their biological impact, *Int. J. Environ. Res. Public Health* 17 (2020) 1–43, <https://doi.org/10.3390/ijerph17165655>.
- [14] A. Gonsioroski, V.E. Mourikes, J.A. Flaws, Endocrine disruptors in water and their effects on the reproductive system, *Int. J. Mol. Sci.* 21 (2020), <https://doi.org/10.3390/ijms21061929>.
- [15] A.C. Gore, V.A. Chappell, S.E. Fenton, J.A. Flaws, A. Nadal, G.S. Prins, R.T. Zoeller, EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals, *Endocr. Rev.* 36 (2015) 1–150, <https://doi.org/10.1210/er.2015-1010>.
- [16] V. Gounden, M. Zain Warasally, T. Magway, R. Naidoo, A. Chuturgoon, A pilot study: bisphenol-A and bisphenol-A glucuronide levels in mother and child pairs in a South African population, *Reprod. Toxicol.* 89 (2019) 93–99, <https://doi.org/10.1016/j.reprotox.2019.07.008>.
- [17] M. Guo, L. Lai, T. Zong, Y. Lin, B. Yang, L. Zhang, H. Kuang, Exposure to di(2-ethylhexyl) phthalate inhibits luteal function via dysregulation of CD31 and prostaglandin F2alpha in pregnant mice, *Reprod. Biol. Endocrinol.* 13 (1) (2015) 11, <https://doi.org/10.1186/s12958-015-0013-4>.
- [18] R.K. Gupta, D.R. Archambeault, H.H.C. Yao, Genetic mouse models for female reproductive toxicology studies, *Compr. Toxicol., Second Ed.* 11 (2010) 561–575, <https://doi.org/10.1016/B978-0-08-046884-6.01135-0>.
- [19] S. Harbison, R. Fleming, Forensic body fluid identification: state of the art, *Res. Rep. Forensic Med. Sci.* 6 (2016) 11, <https://doi.org/10.2147/rrfms.s57994>.
- [20] L.M. Iribarne-Durán, S. Domingo-Piñar, F.M. Peinado, F. Vela-Soria, I. Jiménez-Díaz, E. Barranco, O. Ocón-Hernández, Menstrual blood concentrations of parabens and benzophenones and related factors in a sample of Spanish women: an exploratory study, *Environ. Res.* 183 (2020), 109228, <https://doi.org/10.1016/j.envres.2020.109228>.
- [21] M. Jacob, D. Chappell, B.F. Becker, Regulation of blood flow and volume exchange across the microcirculation, *Crit. Care* 20 (2016) 319, <https://doi.org/10.1186/s13054-016-1485-0>.
- [22] C. Kamunda, M. Mathuthu, M. Madhuku, Health risk assessment of heavy metals in soils from witwatersrand gold mining basin, South Africa, *Int. J. Environ. Res. Public Health* 13 (7) (2016), <https://doi.org/10.3390/ijerph13070663>.
- [23] M. Kumar, D.K. Sarma, S. Shubham, M. Kumawat, V. Verma, A. Prakash, R. Tiwari, Environmental endocrine-disrupting chemical exposure: role in non-communicable diseases, *Front. Public Health* 8 (2020), <https://doi.org/10.3389/fpubh.2020.553850>.
- [24] K. Li, M. Liszka, C. Zhou, E. Brehm, J.A. Flaws, R.A. Nowak, Prenatal exposure to a phthalate mixture leads to multigenerational and transgenerational effects on uterine morphology and function in mice, *Reprod. Toxicol.* 93 (2020) 178–190, <https://doi.org/10.1016/j.reprotox.2020.02.012>.
- [25] X. Li, W. Zhang, J. Lv, W. Liu, S. Sun, C. Guo, J. Xu, Distribution, source apportionment, and health risk assessment of phthalate esters in indoor dust samples across China, *Environ. Sci. Eur.* 33 (1) (2021) 19, <https://doi.org/10.1186/s12302-021-00457-3>.
- [26] Y. Li, G. Huang, H. Gu, Q. Huang, C. Lou, L. Zhang, H. Liu, Assessing the risk of phthalate ester (PAE) contamination in soils and crops irrigated with treated sewage effluent, *Water* 10 (8) (2018), <https://doi.org/10.3390/w10080999>.
- [27] M. Heshmati, Human health consequences of endocrine-disrupting chemicals, *Environ. Issues Sustain. Dev.* (2021), <https://doi.org/10.5772/intechopen.94955>.
- [28] D. Martino, S. Prescott, Epigenetics and prenatal influences on asthma and allergic airways disease, *Chest* 139 (2011) 640–647, <https://doi.org/10.1378/chest.10-1800>.
- [29] L. Niu, Y. Xu, C. Xu, L. Yun, W. Liu, Status of phthalate esters contamination in agricultural soils across China and associated health risks, *Environ. Pollut.* 195 (2014) 16–23, <https://doi.org/10.1016/j.envpol.2014.08.014>.
- [30] E.D.A. Owusu, A. Campillo, J. Daily, X.C. Ding, Acceptance and perceived value of non-invasive malaria diagnostic tests in malaria-endemic countries, *Malar. J.* 20 (1) (2021) 379, <https://doi.org/10.1186/s12936-021-03911-y>.
- [32] O.A. Rotimi, T.D. Olawole, O.C. De Campos, I.B. Adelani, S.O. Rotimi, Bisphenol A in Africa: a review of environmental and biological levels, *Sci. Total Environ.* 764 (2021), <https://doi.org/10.1016/j.scitotenv.2020.142854>.
- [33] S. Sabbaj, Z. Hel, H.E. Richter, J. Mestecky, P.A. Goepfert, Menstrual blood as a potential source of endometrial derived CD3+ T cells, *PLOS One* 6 (12) (2011), e28894, <https://doi.org/10.1371/journal.pone.0028894>.
- [34] M.J. Silva, N.A. Malek, C.C. Hodge, J.A. Reidy, K. Kato, D.B. Barr, J.W. Brock, Improved quantitative detection of 11 urinary phthalate metabolites in humans using liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* 789 (2) (2003) 393–404, [https://doi.org/10.1016/S1570-0232\(03\)00164-8](https://doi.org/10.1016/S1570-0232(03)00164-8).
- [35] R. Smolders, K.W. Schramm, M. Nickmilder, G. Schoeters, Applicability of non-invasively collected matrices for human biomonitoring, *Environ. Health: A Glob. Access Sci. Source* Vol. 8 (2009) 8, <https://doi.org/10.1186/1476-069X-8-8>.
- [36] S.A. Stout, J. Lin, N. Hernandez, E.P. Davis, E. Blackburn, J.E. Carroll, L.M. Glynn, Validation of minimally-invasive sample collection methods for measurement of telomere length, *Front. Aging Neurosci.* 9 (DEC) (2017), <https://doi.org/10.3389/fnagi.2017.00397>.
- [37] Z.R. Tang, X.L. Xu, S.L. Deng, Z.X. Lian, K. Yu, Oestrogenic endocrine disruptors in the placenta and the fetus, *Int. J. Mol. Sci.* 21 (2020), <https://doi.org/10.3390/ijms21041519>.

- [38] U.S. EPA. 2015. Exposure Factors Handbook 2011 Edition (Final Report). U.S. Environmental Protection Agency, (September), 15–21.
- [39] UNEP. 2022. Visual Feature | Beat Plastic Pollution. Retrieved September 22, 2022, from UNEP website: (<https://www.unep.org/interactives/beat-plastic-pollution/?gclid>).
- [40] USEPA. (1995). 40 CFR (7–1-95 Edition) Part 136, Appendix B: USEPA definition and procedure for the determination of the method detection limit. In Code of Federal Regulations.
- [41] K. Usui, Y. Hayashizaki, M. Hashiyada, M. Funayama, Rapid drug extraction from human whole blood using a modified QuEChERS extraction method, *Leg. Med.* 14 (6) (2012) 286–296, <https://doi.org/10.1016/j.legalmed.2012.04.008>.
- [42] L. Wang, M. Liu, W. Tao, W. Zhang, L. Wang, X. Shi, X. Li, Pollution characteristics and health risk assessment of phthalate esters in urban soil in the typical semi-arid city of Xi'an, Northwest China, *Chemosphere* 191 (2018) 467–476, <https://doi.org/10.1016/j.chemosphere.2017.10.066>.
- [43] Y. Wang, H. Zhu, K. Kannan, A review of biomonitoring of phthalate exposures, *Toxics* 7 (2019), <https://doi.org/10.3390/TOXICS7020021>.
- [44] H. Yang, B. Zhou, M. Prinz, D. Siegel, Proteomic analysis of menstrual blood, *Mol. Cell. Proteom.* 11 (10) (2012) 1024–1035, <https://doi.org/10.1074/mcp.M112.018390>.
- [45] A.M. Zama, M. Uzumcu, Epigenetic effects of endocrine-disrupting chemicals on female reproduction: an ovarian perspective, *Front. Neuroendocrinol.* 31 (2010) 420–439, <https://doi.org/10.1016/j.yfrne.2010.06.003>.
- [46] T. Zhang, H. Sun, K. Kannan, Blood and urinary bisphenol a concentrations in children, adults, and pregnant women from China: partitioning between blood and urine and maternal and fetal cord blood, *Environ. Sci. Technol.* 47 (9) (2013) 4686–4694, <https://doi.org/10.1021/es303808b>.
- [47] Q. Zhu, J. Jia, K. Zhang, H. Zhang, C. Liao, G. Jiang, Phthalate esters in indoor dust from several regions, China and their implications for human exposure, *Sci. Total Environ.* 652 (2019) 1187–1194, <https://doi.org/10.1016/j.scitotenv.2018.10.326>.