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RESEARCH ARTICLE

SPE-HPLC-UV Analysis of Phthalates in Biological Fluids of Transfused Mothers in Sacred-Heart Hospital Lantoro Abeokuta Nigeria

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Abstract

Exposure to phthalates through blood transfusion has been a concern of research, as studies have been inconclusive on their leaching of phthalates to the blood of the transfused patient. This study examined levels of diethyl phthalate (DEP), dipropyl phthalate (DPP), dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP) and monobutyl phthalate (MBP) in blood serum, urine and breast milk samples of transfused mothers in Sacred Heart Hospital Lantoro Abeokuta Southwest Nigeria. Samples were extracted using liquid-liquid extraction and cleanup with solid phase extractor (SPE) and thereafter analysed by HPLC-UV. Levels of DEP were 0.062 ± 0.008, 0.012 ± 0.004 and 0.022 ± 0.001 µg/mL in blood serum, urine and breast milk respectively. The DPP levels were 0.070 ± 0.003, 0.005 ± 0.001, and 0.042 ± 0.004 µg/mL respectively. DBP were 0.110 ± 0.004, 0.030 ± 0.020, and 0.200 ± 0.050 µg/mL respectively. Levels of DEHP were 0.099 ± 0.003, 0.016 ± 0.001, and 0.144 ± 0.010 µg/mL respectively. MBP was not detected (ND) in any sample. Estimated daily intake of the phthalates by babies in mothers' breast milk for DEP, DPP, DBP and DEHP were 3.44 µg/kg-bw/day, 6.56 µg/kg-bw/day, 31.25 µg/kg-bw/day, and 22.50 µg/kg-bw/day respectively. Phthalates exposure in transfused mothers was confirmed in this study and that babies were exposed to phthalates through breast milk of mothers.

Keywords

Phthalates, Blood transfusion, Biological fluids, Exposure, Daily intake

Introduction

Phthalates are esters of phthalic acid, they are used as plasticizers in plastics, drug capsule coatings, medical devices, food packaging, cosmetics, plastic toys, furniture, car upholstery, cellulose acetate plastics, latex adhesives, nail polish, sealants, vinyl tile, carpet tile and artificial leathers [1,2]. Overtime, they leach extensively from these materials into the content and the immediate environment. A scientific study had reported that they make up to sixty percent (60%) of these materials [3], however, they are not chemically bonded to these materials thus leading to extensive leaching into the immediate environment [4]. Hence, phthalates have been detected in soil, air, water sediments, medical equipment, amniotic fluids, umbilical cord, sweat etc. It has been well established that phthalates are the most used plasticizer in polymers [5].

Some scientific research studies have suggested that there is a strong link between *in-utero* exposure and reduced anogenital distance (AGD), a marker of reduced androgen exposure and potential indicator of reduced human male fertility in adult, preterm birth, reduced intellectual and motor development in children, as well as respiratory effects, increased waist circumference and

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insulin resistance [1,6-8]. A recent study reported that prenatal exposure definitely reduced the total and free testosterone/luteinizing hormone ratio in human male offspring [5]. Dibutyl phthalate (DBP) and Diethylhexyl phthalate (DEHP) have been associated with reduced anogenital distance in 2-6 year-old male children, and MBP in amniotic fluid has been associated with shorter anogenital distance in female [1]. Furthermore, DEHP has been shown to promote tumor growth frequency in animals as well as testicular tubular atrophy when given in dose [9]. Thus, phthalates have been classified as endocrine disrupting chemical which alter some functions of the endocrine system and having adverse effect in the living organism [10]. The routes of exposure include inhalation, ingestion and skin absorption and intravenous injection [11].

A research study reported a 12 fold increase in DEHP concentration in whole blood stored for 42 days and a 20 fold increase in Mono-(2-ethylhexyl) phthalate (MEHP) in whole blood stored for 42 days [12]. Study have reported increase in DEHP concentration in blood plasma stored at 4 °C for one week from 100 mg/L to 275 mg/L, and an increase in DEHP concentration in platelets and plasma stored at ambient temperature for 3 days from 200 mg/L to 300 mg/L [13]. A French study found fourteen (14) phthalates in medical PVC devices of which DEHP was found in significant quantity [14]. However, little attention is given to the phthalates exposure through blood transfusion in medical care, most especially mothers who during parturition are usually in dire need of blood to maintain safe blood level and therefore are transfused with blood. The migration of phthalates into blood stream through blood transfusion is the most lethal as it delivers the phthalates directly into the blood stream of the transfused patient.

There has been paucity of scientific data on the level of phthalates and estimate of daily intake of phthalate in blood, urine and breast milk samples of transfused mothers most especially in Abeokuta, Southwest Nigeria. Also, inappropriate extraction and cleanup procedure usually makes the scientific results doubtful and unreliable. Hence, this study aimed to examine the level of phthalates in blood, urine and breast milk samples of transfused mothers in Sacred Heart Hospital Lantoro Abeokuta using liquid-liquid extraction and solid phase extractor (SPE) cleanup and thereafter analysed by HPLC-UV. The estimate of the daily intake of phthalates by the babies of the transfused mothers was calculated. The daily intake was estimated from the concentration of the phthalates obtained in breast milk samples.

Materials

The analytical standards namely diethyl phthalate, dipropyl phthalate, dibutyl phthalate, diethylhexyl phthalate monobutyl phthalate and benzyl benzoate as well as HPLC grade dichloromethane, ethyl acetate, acetonitrile, n-hexane, diethyl ether, sodium chloride, sodium carbonate, were purchased from Merck Germany. J & K scientific C-18 solid phase extractor cartridges were obtained from J & K Scientific LLC. U.S.A.

Ethical statement

This study obtained ethical approval from Sacred Heart Hospital Lantoro Abeokuta ethical committee (Ethical number: SHH/EC/EA/01/02/19). Written informed consent form was endorsed by each of the recruited mother after full understanding of the research protocol and thereafter data collection.

Sample collection

Informed consent form was signed by each recruited transfused mother and the questionnaire was administered before the samples were collected from the mothers. Two (2) transfused mothers consented to the study and their blood, urine and breast milk samples were collected from them within 72 hours of blood transfusion of the mothers. Urine, blood and breast milk samples were collected by medical doctor. Each blood sample was collected into 5 mL bijou bottle and was centrifuged at 4000 rpm for 15 minutes to obtain the serum, this was done within 4 hours of collection of the blood sample, and 1 mL of 0.1M H_3PO_4 was added to the serum to prevent enzymatic hydrolysis and was frozen at -20 °C in the freezer till the time of analysis. The urine was collected into 30 mL universal bottle 1 mL of 0.1M H_3PO_4 was added to the urine to prevent enzymatic hydrolysis and was kept frozen at -20 °C in the freezer till the time of analysis. The breast milk was hand expressed by each recruited mother into 5 mL bijou bottle after abstaining from personal care products and proper hand washing, thereafter, 1 mL of 0.1M H_3PO_4 was added to the breast milk to prevent enzymatic hydrolysis and was frozen at -20 °C in the freezer till the time of analysis.

Extraction of blood serum samples

A slightly modified serum or urine extraction procedure of Kondo, et al. [15] and Ogunfowokan, et al. [16] was employed, in which 3 mL serum was put in a 25 mL test tube, and 1 µg mL^{-1} benzyl benzoate internal standard was added as spike addition. In order to coagulate the amino acids in plasma or serum, 1 mL of acetonitrile was added to the sample. The sample was then extracted using 3 × 5 mL of dichloromethane:hexane (12.5:87.5 v/v) in an ultrasonic bath. The extract was pooled, and was further washed with 3 × 5 mL of 0.1M sodium carbonate. The combined extract was then concentrated at ambient temperature to 2 mL before cleanup.

Extraction of urine samples

The urine extraction was modified procedure of Kondo, et al. [15] and Ogunfowokan, et al. [16], in which 5 mL of urine sample was placed in a test tube and 1 µg

mL^{-1} benzyl benzoate internal standard was added to the sample as spike and sodium chloride (1g) was added to the sample, before being extracted in an ultrasonic bath with 3 × 5 mL of dichloromethane:hexane (12.5:87.5 v/v). The extracts were combined, and the pooled extract was washed with 3 × 5 mL of 0.1M sodium carbonate, after which, the pooled extract was evaporated at ambient temperature to 2 mL for cleanup.

Extraction of breast milk samples

The extraction of breast milk was a modification of procedure by Sorensen [17] and Ogunfowokan, et al. [16]. In a test tube with 5 mL of milk sample, a benzyl benzoate internal standard of 1 µg mL^{-1}, was added. The milk sample was then extracted in an ultrasonic bath with 3 × 5 mL of diethyl ether:hexane (50:50 v/v) the extracts were pooled and the pooled extract washed with 3 × 5 mL of 0.1M sodium carbonate. The extract was concentrated at ambient temperature to 2 mL prior to cleanup, and it underwent the same procedures as previous samples.

Solid Phase Extractor cleanup of extract

The cleanup procedure was a modification of Olujimi, et al. [18] method in which C-18 J & K Scientific cartridges containing 500 mg/6 mL was loaded with the sample extract using glass syringe. It was conditioned with n-hexane and later eluted with 2 mL of ethyl acetate and then 2 mL of acetonitrile. The eluate was evaporated to dryness and reconstituted into 1 mL with acetonitrile and stored at -20 °C until analysis.

Estimation of the daily intake from breast milk phthalate concentration

The estimate of phthalate daily intake through the breast milk was calculated using the model used by Kim, et al., [19] with slight modification, in the following equation was used.

Daily intake (µg/kg bw-day) =

$$\frac{MC(\mu g/L) \times DBI\ (mL)}{1000 \times body\ weight\ (kg)}$$

Where MC = The breast milk concentration of phthalate;

DBI = The daily breast milk intake quantity of 3-4 days old babies (500 mL),

Baby weight mean = 3.2 kg

In the literature the ratio of parent phthalate to metabolite for any specific phthalate in breast milk is 0.11 [20,21]. The daily intake was calculated.

Analysis using HPLC

On Agilent Technology HPLC 1100 series equipped with a UV detector and a WatersX bridge C18 100 × 4.6 mm, 3.5 µm column, each cleanup extract was analyzed twice. Acetonitrile:water (90:10 v/v) was the mobile phase and the working temperature was 30 °C, while the pressure was 99 bar. The flow rate was 1.0 mL per minute, the injection volume was 20 µL, The wavelength the phthalates and the metabolites were identified was 226 nm. The retention time of MBP, DEP, DPP, DBP and DEHP were 4.135, 9.675, 11.468, 13.291 and 21.758 minute respectively. The peak area against concentration of the mixed standards was used to obtain the calibration curve on Microsoft Excel 2010 and the concentration of each sample was calculated from the calibration curve.

Linearity and sensitivity

Seven (7) point calibration curve in the range 0.2 to 20 µg/mL of each phthalate internal standard was used to obtain the linear calibration curve of the HPLC. The slope of each phthalate was used to calculate the concentration of the phthalate in the sample. The regression coefficient for MBP, DEP, DPP, DBP and DEHP were 0.9996, 0.9996, 0.9995, 0.9995 and 0.9966 respectively.

Results and Discussion

Trends in phthalates levels

The trend in the phthalate concentration in this study shown in Table 1 was DEP; blood serum > breast milk > urine, while DPP; blood serum > breast milk > urine, also DBP; breast milk > blood serum > urine, and DEHP; breast milk > blood serum > urine, but MBP; blood serum ≡ breast milk ≡ urine. This suggests that the transfused mothers were exposed to phthalates through the blood transfusion.

The frequency of detection of DEP in serum in this study was 100% (Table 1), also, the DEP in serum was in the range 0.053 to 0.071 µg/mL, and the mean was 0.062 ± 0.008 µg/mL (Arithmetic mean ± standard deviation), this was higher than ND (not detected) reported in our earlier study in non-transfused mothers (control samples) in Ibadan [22], this suggests exposure to phthalate through blood transfusion in the transfused mothers. This is higher than that reported in a Swedish study by Hogberg, et al., [21], which reported a mean of 0.31 ± 0.26 ng/mL (Table 2). It is also lower than 0.23 ± 0.28 µg/mL and 0.024 ± 0.046 µg/mL respectively reported in our earlier and recent studies in Hospitals in Ibadan [22,23]. The urinary frequency of detection of DEP in this study was 100%, and the range of DEP in urine in this study was 0.007 to 0.016 µg/mL, and the mean was 0.012 ± 0.004 µg/mL this is higher than ND (not detected) reported in control samples that is non-transfused mothers in our earlier study in Ibadan [22]. It suggests that phthalate exposure from blood bags to blood of the transfused mothers, which was excreted in the urine. The mean DEP in urine in this study is higher than range of mean values between ND to 1.13 ± 0.10

Table 1: Lantoro Hospital phthalates and metabolite levels in serum, urine and breast milk compared with literature level.

Analyte	LOD	Sample	Quantity (µg mL^{-1})									
			Results				A. means ± SD (This Study)	GM	Median	Min.	Max	FOD%
			1	2	3	4						
DEP	0.01	Serum	0.071	0.070	0.053	0.055	0.062 ± 0.008	0.060	0.060	0.053	0.071	100
		Urine	0.007	0.008	0.016	0.015	0.012 ± 0.004	0.011	0.011	0.007	0.016	100
		B. milk	0.022	0.023	ND	ND	0.022 ± 0.001	0.020	0.020	ND	0.023	50
DPP	0.007	Serum	0.067	0.064	0.071	0.073	0.070 ± 0.003	0.070	0.070	0.064	0.073	100
		Urine	0.005	0.005	ND	ND	0.005 ± 0.001	0.005	0.005	ND	0.005	50
		B. milk	0.038	0.039	0.046	0.043	0.042 ± 0.004	0.040	0.040	0.038	0.046	100
DBP	0.007	Serum	0.112	0.108	ND	ND	0.110 ± 0.004	0.110	0.110	ND	0.112	50
		Urine	0.007	0.006	0.056	0.051	0.030 ± 0.020	0.029	0.029	0.006	0.056	100
		B. milk	0.147	0.150	0.271	0.231	0.200 ± 0.050	0.182	0.190	0.147	0.270	100
DEHP	0.007	Serum	0.101	0.096	ND	ND	0.099 ± 0.003	0.098	0.098	ND	0.101	50
		Urine	0.017	0.015	ND	ND	0.016 ± 0.001	0.016	0.016	ND	0.017	50
		B. milk	0.155	0.132	ND	ND	0.144 ± 0.010	0.143	0.143	ND	0.155	50
MBP	0.007	Serum	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
		Urine	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
		B. milk	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Keys: LOD: Limit of Detection; A. Mean ± SD: Arithmetic Mean ± Standard Deviation; GM: Geometric Mean; Min: Minimum Value; Max: Maximum Value; ND: Not Detected; B. Milk: Breast Milk: FOD: Frequency of Detection

Table 2: Lantoro Hospital phthalates and metabolite levels in serum, urine and breast milk compared with literature level.

Analyte	Sample	A. means ± SD (This study)	Lit. level	Author	Nation
DEP	Serum	0.062 ± 0.008	0.31 ± 0.26 ng/mL	Hogberg, et al. [21]	Sweden
	Urine	0.012 ± 0.004	ND- 1.13 ± 0.10 µg/L	Adenuga, et al. [24]	Nigeria
	B. milk	0.022 ± 0.001	0.30 ± 0.24 ng/mL	Hogberg, et al. [21]	Sweden
DPP	Serum	0.070 ± 0.003	0.07 ng/mL	Zhang, et al. [25]	China
	Urine	0.005 ± 0.001	0.12 ng/mL	Zhang, et al. [25]	China
	B. milk	0.042 ± 0.004	0.03 ng/mL	Zhang, et al. [25]	China
DBP	Serum	0.110 ± 0.004	1.20 ± 1.60 ng/mL	Hogberg, et al. [21]	Sweden
	Urine	0.030 ± 0.020	ND - 1.20 ± 0.14 µg/L	Adenuga, et al. [24]	Nigeria
	B. milk	0.200 ± 0.050	2.80 ± 3.40 ng/mL	Hogberg, et al. [21]	Sweden
DEHP	Serum	0.099 ± 0.003	5.90 ± 21.00 ng/mL	Hogberg, et al. [21]	Sweden
	Urine	0.016 ± 0.001	ND – 5.51 ± 0.38 µg/L	Adenuga, et al. [24]	Nigeria
	B. milk	0.144 ± 0.010	17.00 ± 47.00 ng/mL	Hogberg, et al. [21]	Sweden
MBP	Serum	ND	1.80 ± 3.30 ng/mL	Hogberg, et al. [21]	Sweden
	Urine	ND	17.47 ± 15.70 ng/mL	Dualde, et al. [26]	Spain
	B. milk	ND	1.20 ± 1.30 ng/mL	Hogberg, et al. [21]	Sweden

Keys: A. mean ± SD: Arithmetic mean ± standard deviation; Lit. level: Literature level; ND: Not Detected; B. milk: Breast milk

µg/L reported by Adenuga, et al., [24] in a Nigerian study. Also, the level of DEP in urine in this study is lower than 0.1 ± 0.09 µg/mL, and 0.004 ± 0.007 µg/mL respectively reported in our earlier studies [22,23]. The mean DEP in breast milk in this study was in the range ND to 0.023 µg/mL, and the mean was 0.022 ± 0.001 µg/mL this is higher than ND (not detected) reported in our earlier study in non-transfused mothers that is control samples in Ibadan [22]. This suggests that DEP exposure through blood transfusion in the transfused mothers got eliminated in the breast milk. The level of DEP in breast milk in our study is higher than 0.30 ± 0.24 ng/mL reported in a Swedish study by Hogberg, et al. [21]. Phthalate burden of DEP of the transfused mother in this study was 0.09 µg/mL. Level of DEP in breast milk in this study is lower than 1.79 ± 1.24 µg/mL, but higher than 0.009 ± 0.026 µg/mL reported in our earlier studies in hospitals in Ibadan [22,23].

Also, DPP in serum in this study was in the range

0.064 to 0.073 µg/mL, while the mean was 0.070 ± 0.003 µg/mL. It gives credence to the suggestion that phthalates leached from blood bags into the blood stream of the transfused mothers. The DPP reported in this study is higher than 0.07 ng/mL reported in a Chinese study by Zhang, et al. [25]. The DPP in serum in this study is lower than 1.8 ± 2.7 µg/mL and 0.401 ± 0.892 µg/mL respectively reported in our earlier and our recent studies [22,23]. Similarly DPP in urine sample in this study was in the range ND to 0.005 µg/mL, and the mean was 0.005 ± 0.001 µg/mL. This was the lowest level of parent phthalate in all the samples analysed in this study. The level of DPP in urine in our study is higher than that declared in Chinese study in which the DPP was 0.12 ng/mL [25]. Whereas, the DPP in urine reported in our earlier and recent studies were 0.22 ± 0.35 µg/mL and 0.055 ± 0.096 µg/mL respectively [22,23]. DPP in breast milk was in the range 0.038 to 0.046 µg/mL, and the mean was 0.042 ± 0.004 µg/mL. The level obtained in our study is higher than 0.23 µg/L reported in a Chinese study by Zhang, et al. [25]. The level of DPP declared in this is lower than 0.76 ± 0.61 µg/mL and 0.111 ± 0.309 µg/mL respectively, reported in our earlier studies [22,23].

Concentration of DBP in serum in this study was in the range ND to 0.112 µg/mL, and the mean was 0.110 ± 0.004 µg/mL, this was the third highest in parent phthalates in all the samples analysed, this is higher than ND we reported in our earlier study in non-transfused mothers (control samples) in Ibadan [22]. This suggests that phthalate leached from, the blood bags to the system of the transfused mothers, since DBP and DEHP are the most important plasticizers used in medical polymer materials [20]. The concentration of DBP in this study Is higher than 1.20 ± 1.60 ng/mL reported in a Swedish study [21]. However, our earlier and recent studies reported higher DBP levels that is 1.3 ± 2.75 µg/mL and 0.933 ± 1.817 µg/mL respectively [22,23].

Level of DBP in urine in this study was in the range 0.006 to 0.056 µg/mL, and the mean was 0.030 ± 0.020 µg/mL. This is higher than range of mean values between ND to 1.20 ± 0.14 µg/L reported by Adenuga, et al. [24] in a Nigerian study. Again, the level reported in this study is lower than 0.15 ± 0.4 µg/L and 0.142 ± 0.239 µg/mL respectively in our earlier and recent studies in Ibadan [22,23]. Breast milk concentration of DBP in this study was in the range 0.147 to 0.271 µg/mL, and the mean was 0.200 ± 0.050 µg/mL. This is the highest level of parent phthalate in all the samples in this study. The level reported in this study is higher than 2.80 ± 3.40 ng/mL reported in a Swedish study by Hogberg, et al. [21]. DBP in our earlier and recent studies were however higher than this study in which we reported 0.80 ± 1.00 µg/mL and 0.335 ± 0.830 µg/mL respectively [22,23].

Level of DEHP in serum in this study was in the range ND to 0.101 µg/mL, and the arithmetic mean was 0.099 ± 0.003 µg/mL, this is higher than ND we reported in non-transfused mothers that is control samples in our earlier study in Ibadan [22]. This suggests that phthalate leached extensively from blood bags to the blood system of the transfused mothers. The level of DEHP in this study is higher than 5.9 ± 21 ng/mL reported in a Chinese study by Hogberg, et al. [21]. However, our recent study reported higher DEHP in serum; 1.108 ± 1.290 µg/mL [22]. Urinary concentration of DEHP in this study was in the range ND to 0.017 µg/mL, and the mean was 0.016 ± 0.001 µg/mL. this is higher than ND (not detected) reported in our earlier study in non-transfused mothers (control samples) in Ibadan [22]. The value of DEHP in urine in this study is higher than range of mean values between ND to 5.51 ± 0.38 µg/L reported in a Nigerian study by Adenuga, et al. [24]. Nonetheless, our recent study reported higher DEHP concentration in urine than this study which was 0.205 ± 0.244 µg/mL [22]. DEHP in breast milk was in the range ND to 0.155 µg/mL, and the mean was 0.144 ± 0.010 µg/mL. This was the second highest level of parent phthalates in all the samples analysed in this study. This is higher than ND (not detected) reported in our earlier study in non-transfused mothers (control samples) in Ibadan [22]. This could suggest that transfusion is a source of exposure to phthalates in transfused patient. The level of DEHP found in this study is higher than 17.00 ± 47.00 ng/mL reported in a Swedish study by Hogberg, et al. [21]. Our recent study reported higher level of DEHP in breast milk and that was 0.457 ± 1.154 µg/mL [22].

Frequency of detection of MBP in serum in this study was 0%, that is MBP was not detected (ND) in the serum samples in this study, and this was the least concentration in the phthalate (metabolite) examined in this study. This could suggest that MBP had been eliminated from the system of the transfused mothers in the study, since it has short half-lives in the human system. The level of MBP in this study is less than 1.80 ± 3.30 ng/mL reported in a Swedish study by Hogberg, et al. [21]. The level of MBP in serum in this study is lower than 1.50 ± 2.10 µg/mL and 0.006 ± 0.011 µg/mL respectively reported in our earlier and recent studies in Hospitals in Ibadan [22,23]. Likewise, the frequency of detection of MBP in urine in this study was 0%, hence, MBP was not detected in urine samples in this study. Urinary level of MBP in this study is the lowest for all urinary phthalate (metabolite) examined in this study. The MBP levels in this study were lower than 12.21 ± 11.61 ng/mL, 17.47 ± 15.71 ng/mL and 34.90 ± 35.44 ng/mL respectively (Median, arithmetic mean and geometric mean) reported in a Spanish study by Dualde, et al. [26]. The level of MBP in urine in this study is lower than 0.70 ± 0.87 µg/mL and 0.002 ± 0.003 µg/mL respectively reported in our earlier and recent studies [22,23]. Similarly, the frequency of detection of MBP in breast milk in the study was 0%, and subsequently MBP was not detected in any of the samples in this study. This

Table 3: Concentration of phthalate in breast milk and estimated daily intake levels.

Phthalate	B. milk Conc. (µg/L)	Estimated daily intake (µg/kg bw-day)
Diethyl phthalate (DEP)	22	3.44
Dipropyl phthalate (DPP)	42	6.56
Dibutyl phthalate (DBP)	200	31.25
Diethylhexyl phthalate (DEHP)	144	22.50

Keys: B. milk: Breast milk; Conc: Concentration

could suggest a recent exposure to phthalates in the mother which had not been excreted from their body system. The MBP level in this study is lower than 1.20 ± 1.30 ng/mL reported in a Swedish study by Hogberg, et al. [21]. The level of MBP in breast milk in this study was lower than our earlier and recent studies which were 5.72 ± 6.60 µg/mL and 0.001 ± 0.003 µg/mL respectively [22,23].

Estimated daily intake from mothers' breast milk

The estimates of the daily phthalates intake by the babies from the mothers' breast milk phthalate concentration are given in Table 3.

The DEP daily intake in this study was 3.44 µg/kg bw-day (Table 3), this is higher than 0.30 µg/kg bw-day for under ten females, and also higher than under ten males, as well as over ten males and females which were 0.5, 1.5 and 1.1 µg/kg bw-day respectively reported in a Chinese study by Guo, et al. [27]. The DEP estimated daily intake in this study is lower than United State Environmental Protection Agency (USEPA) oral reference of 800 µg kg^{-1} d^{-1} established on no observed effect level (NOEL) of 750 mg/kg-bw/day [28], as well as the WHO total daily intake of 500 µg/kg body weight per day [29]. Also DPP daily intake in this study was 6.56 µg/kg bw-day, this suggests the newborns in this study were exposed to phthalate through their mothers' breast milk.

The DBP daily intake in this was 31.25 µg/kg bw-day, this is higher than 0.65 µg/day reported in a Canadian study by Zhu, et al. [30]. This suggests the babies in this study could be exposed to phthalate through their mothers' breast milk. The DBP daily intake in this study is higher than the European Food Safety Authority (EFSA) permissible limit of 10 µg/kg bw-day, but lower than USEPA reference dose of 100 µg/kg bw-day [29,31]. This study reported DEHP daily intake of 22.50 µg/kg bw-day, this is higher than the range of 0.91-6.52 µg/kg bw-day of daily intake reported in a Korean study by Kim, et al. [19]. This suggests that the babies were exposed to phthalates through the breast milk. The DEHP estimated daily intake in this study is lower than EFSA limit of 50 µg/kg bw/day, as well as USEPA reference dose of 100 µg/kg bw/day [29].

Conclusion

This study affirmed that DEP, DPP, DBP and DEHP leached into the blood system and by extension urine and breast milk of the transfused mothers, though the metabolite MBP was below the detection limit in all the samples. The estimated daily intake also showed similar trend in which substantial phthalates were ingested by the babies through their mothers' breast milk. Nonetheless, the levels found in this study were not higher than the permissible limits set by EFSA, USEPA and WHO, except DBP that was higher than EFSA limit. This suggests that the phthalate levels were not at the hazard level for adult humans, but could be extremely toxic to babies exposed to phthalates through the mothers' breast milk. Besides, cumulative exposure through other environmental exposure could raise the level above the permissible limit. Hence, we suggest a less harmful substitute of phthalate should be sought and used as plasticizers in transfusion devices.

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Conflict of Interest

The authors declare no conflict of interest.

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Statement of Authors' Equal Contribution

We declare that authors have contributed significantly equally to this research and the manuscript.

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