Prenatal exposure to persistent organic pollutants in Northern Tanzania and their distribution between breast milk, maternal blood, placenta and cord blood.

Müller MHB[1]*, Polder A[1], Brynildsrud OB[2], Grønnestad R[1,8], Karimi M[1], Lie E[1,4], Manyilizu WB[5], Mdegela RH[5], Mokiti F[6], Murtadha M[6], Nonga HE[5], Skaare JU[7], Solhaug A[7], Lyche JL[1]

[1]Norwegian University of Life Sciences, Campus Adamstuen, P.O. Box 8146 Dep, N- 0033 Oslo, Norway

[2]Norwegian Institute of Public Health, P.O. Box 4404 Nydalen, N-0403 Oslo

[4]Norwegian Institute for Water Research, Gaustadallèen 21, N-0349 Oslo, Norway

[5]Sokoine University of Agriculture, Department of Veterinary Medicine and Public Health, P.O. Box 3021, Morogoro, Tanzania

[6]Mount Meru Regional Referral Hospital, P.O. Box 3092, Arusha, Tanzania

[7]Norwegian Veterinary Institute, P.O. Box 750 Sentrum, N-0106 Oslo, Norway

[8]Norwegian University of Science and Technology, NO-7491 Trondheim, Norway

**Corresponding author:* Norwegian University of Life Sciences, Campus Adamstuen, P.O. Box 8146 Dep, N- 0033 Oslo, Norway. Tel.: +47 94815141; Fax: +47 22597089

Email address: mette.helen.bjorge.muller@nmbu.no

Keywords

POPs, Prenatal exposure, Breast milk transfer, Placental transfer, Tanzania

Abstract

Human exposure to persistent organic pollutants (POPs) begins during pregnancy and may cause adverse health effects in the fetus or later in life. The present study aimed to assess prenatal POPs exposure to Tanzanian infants and evaluate the distribution of POPs between breast milk, maternal blood, placenta and cord blood. For assessment of prenatal exposure, 48 maternal blood samples from Mount Meru Regional Referral Hospital (MMRRH), Arusha Tanzania, were analyzed for organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), dioxin-like (DL) activity and perfluorinated alkyl substances (PFASs). For evaluation of POPs distribution between maternal/infant compartments, breast milk, placenta and cord blood corresponding to the maternal blood were analyzed for OCPs, PCBs and BFRs. In maternal blood, p,p´- DDE was detected in 100% of the samples ranging between 29 and 1890 ng/g lipid weight (lw). PCB-153 was the only PCB detected in maternal blood, with detection rate of 29% and concentrations up to 116 ng/g lw. BDE-47 was detected in 65% of the maternal blood samples, ranging between <LOD and 83.2 ng/g lw. DL activity was measured using Dioxin Responsive CALUX® bioassay. The DL activity was above LOQ in 92% of the samples, ranging from <LOQ to 114 pg CALUX TEQ/g lw. PFASs was dominated by PFOS and PFOA, however, the concentrations were low (range ∑PFASs 0.18-3.14 ng/mL). p,p´-DDE was detected in 100% of the breast milk, placenta and cord blood samples and the concentrations were strongly correlated (r=0.89-0.98) between all compartments. Maternal blood (MB) had significantly lower p,p´-DDE concentrations (ng/g lw) than cord blood (CB) and breast milk (BM). The median CB/MB ratio was 1.3 and median MB/BM ratio was 0.8. p,p´-DDE concentrations in breast milk and cord blood did not show significant difference and median CB/BM ratio was 1. In addition, the relative p,p`-DDE transfer from maternal blood to breast milk and to cord blood increased when p,p`-DDE concentrations in maternal blood increased. This study shows that Tanzanian infants are exposed to a wide range of POPs during fetal life, which raise concerns for potential health effects. In addition, this study found that maternal blood concentrations may lead to underestimation of prenatal exposure, while breast milk collected close to delivery may be a more suitable indicator of prenatal exposure.

Keywords

POPs, Prenatal exposure, Breast milk transfer, Placental transfer, Tanzania

Funding

This study received financial support from the Research Council of Norway (NFR nr: 204051).

Ethics

The mothers were informed about the project in English and Swahili and gave informed, written consent prior to enrolment. A personal identification code was given to each mother and the confidentiality was taken care of. This project is ethically approved by the Medical Research Coordinating Committee of the National Institute for Medical Research, Tanzania (Ref: NIMR/HQ/R.8a/Vol.IX/1354), and Regional Committee for Medical and Health Research Ethics, Section South East C, Norway (Ref: IRB 1870).

1. Introduction

Human exposure to persistent organic pollutants (POPs), like organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), dioxins and perfluorinated alkyl substances[1] (PFASs), are inevitable as POPs are ubiquitously present in the global environment. POPs have benefited humans through enhancement of agricultural production, vector control and industrial productivity until their toxic, persistence and bioaccumulative properties became identified. Since the 1970s, an expanding number of chemicals has been identified as POPs and included in The Stockholm Convention of Persistent Organic Pollutants (POPs), which was established to prevent production and discharge of POPs into the environment (The Stockholm Convention 2016). Nevertheless, in developing countries, such as Tanzania, use and discharge of POPs are still present from increasing industrial and agricultural activities, and poor waste management (Asante et al. 2011; Kaseva et al. 2002; Ntow et al. 2008). Tanzania ratified the Stockholm Convention in 2004. However, the documentation of POP sources and human exposure in Tanzania, as well as other developing countries, is limited (UNEP 2009).

Worldwide, populations are mainly exposed to POPs through food, household consumer products and dust (EFSA 2007, 2012; Eriksson and Karrman 2015; Lorber 2008). However, populations in developing countries may have additional risks of POPs exposure from pesticide spraying during vector control or farming, industrial occupations, obsolete pesticide storages and improper waste disposals (Chen et al. 2015; Darnerud et al. 2006; Kannan et al. 1997; Polder et al. 2010; Van Dyk et al. 2010).

Human exposure to POPs begins during prenatal life through placental transfer and continues postpartum through breastfeeding. The placenta is a semipermeable barrier separating the maternal and the fetal blood circulation, and regulates the exchange of nutrients, gases, waste and endogenous as well as exogenous molecules, like POPs (Prouillac and Lecoeur 2010). The ability of POPs to be transferred from mother to fetus through placenta and mother to infant through breastfeeding are well-known (Dewan et al. 2013; Skaare et al. 1988). The concentrations and timing of POPs exposure during prenatal life are risk factors for the potential of causing adverse health effects. Fetuses and infants may be more vulnerable to POPs exposure than adults as POPs

[1] PFOS and PFOA are included in the Stockholm Convention, but other PFASs are not defined as POPs.

may disrupt developmental processes, which further can lead to permanent changes in organs and physiological systems. Furthermore, developing individuals may accumulate higher concentrations and prolong the excretion of POPs due to immature metabolic capacity (WHO 2010). Increased attention is drawn to the suggested associations between prenatal exposure to POPs and developmental, neurological, reproductive and immunological impairments, as well as diseases later in life, like cancer, obesity and diabetes (Cohn et al. 2007; Cooper et al. 2004; Dalvie et al. 2004; Dewan et al. 2013; Eskenazi et al. 2006; Langer 2010). Despite the scientific focus on assessments of prenatal POPs exposure, there is a lack of standardization between studies regarding subject selection, timing of sampling and reported concentrations, i.e. wet weight, lipid weight, in plasma, serum or whole blood (Jakobsson et al. 2012). Principally, POPs passes cell membranes via passive diffusion and distribute depending on the lipid content of the compartment. However, chemical properties like molecular weight, chemical structure and protein binding may influence the distribution. Dewan et al. (2013) suggested that some OCPs may selectively partition into breast milk, while other OCPs may be transferred through placenta. It is reported that placental transport of PBDE congeners decrease with increasing bromination (Frederiksen et al. 2010). Another study found that PFOA pass through the placenta easier than PFOS and PFHxS (Midasch et al. 2007), and branched isomers of PFOS pass more easily than the linear isomer (Hanssen et al. 2010). Furthermore, 1,2,3,7,8-PentaCDD and 2,3,4,7,8-PentaCDF are shown to accumulate to a higher extent in the placenta compared to other dioxin congeners, while PCDDs cross the placental barrier more readily than PCDFs and non-ortho PCBs (Tsukimori et al. 2013). The impact of degree and positioning of chlorination, bromination or fluorination, as well as the transport mechanisms of POPs between maternal and fetal/infant compartments are still not fully understood (Barr et al. 2005; Vizcaino et al. 2014).

This study is a part of a mother-infant exposure assessment study within the MORATANZ[2] project, performed in Arusha, Tanzania. Previously, we have assessed the postnatal exposure to OCs and BFRs through breast milk for 95 nursing infants. The estimated daily intake of DDTs, dieldrin, nondioxin-like (NDL) PCBs and polybrominated diphenyl ethers (PBDEs) exceeded the health based guidance values in 2%, 6%, 50% and 20% of the nursing infants, respectively (Müller et al.

[2] Monitoring and Risk Assessment of Contaminants in Southern Africa: using Arusha in Tanzania as a model.

2016; Müller et al. 2017). The present study aimed to assess the prenatal exposure to OCPs, PCBs, BFRs, DL compounds and PFASs in Tanzanian infants. In addition, distribution of POPs between breast milk, maternal blood, placenta and cord blood was evaluated to increase the knowledge of mother-infant transfer of POPs. Furthermore, the suitability of using breast milk or maternal blood concentrations for determining prenatal exposure was assessed.

2. Materials and methods

2.1 Sample collection and selection

Breast milk, maternal blood, placenta and cord blood were collected from 150 healthy, primiparous mothers at Mount Meru Regional Referral Hospital (MMRRH), Arusha Tanzania, between October and December 2012. Characteristics of the mothers and infants were recorded by means of a questionnaire. The questionnaire was prepared using WHO guidelines (WHO 2007) and adjusted for this study. After delivery, maternal blood and cord blood were collected into EDTA vacutainers. After centrifugation, plasma was transferred to labeled cryo vials and stored in liquid nitrogen. Placenta tissue was collected into sterile, polypropylene tubes and stored at -20º C. At least 10 mL of maternal plasma and cord plasma, and 5x5 cm of placenta were requested. The collection of breast milk is described in Müller et al. (2016). The samples were transported by World Courier® AmerisourceBergen on dry ice to Norway and kept frozen at -20º C until analysis.

The selection of samples is illustrated in *Fig*. S1. Of the 150 mother/infant couples included in the project, 48 maternal blood samples were selected for prenatal exposure assessment based on the sample amount (>4 grams). One of the 48 questionnaires was excluded due to a sample code error. For assessment of distribution, the corresponding breast milk (N=47), placenta (N=45) and cord blood samples (N=46) were included. Samples <1 gram were excluded. The breast milk samples are also included in Müller et al. (2016) and Müller et al. (2017).

2.2 Chemical analyses

The chemical analyses were performed at the Laboratory of Environmental Toxicology, Norwegian University of Life Sciences (NMBU), Oslo, Norway. The laboratory is accredited by the Norwegian Accreditation for chemical analysis of OCPs, PCBs and BFRs in human samples according to the requirements of the NS-EN ISO/IEC 17025 (TEST 137). Dioxin-Responsive Chemically Activated Luciferase eXpression (DR CALUX®) bioassay and PFAS analyses are not included in this accreditation, but they are validated according to the same procedures.

Breast milk, maternal blood, placenta and cord blood were analyzed for organochlorine pesticides (OCPs) group I and PCBs: hexachlorobenzene (HCB), β- and γ-hexachlorocyclohexanes (ΣHCHs), oxychlordane and trans-nonachlor (ΣCHLs), bis-2,2-(4-chlorophenyl)-1,1,1-trichloroethane (p,p′-DDT) and its metabolites p,p′-DDE, o,p′-DDD, p,p′-DDD, and o,p′-DDT (ΣDDTs), mirex, toxaphene congeners (ΣCHBs) Parlar #: CHB-26, -40, -41, -44, -50 and -62; PCBs (Σ_7PCBs): IUPAC #: PCB-28, -52, -101, -118, -138, -153 and -180; OCPs group II: heptachlor, cis-heptachlor epoxide, trans-heptachlor epoxide, dieldrin, endrin, tecnazen*, quintozen*, pentachloranilin*, α- and β-endosulfan and endosulfan sulphate, BFRs[3]: PBDEs, hexabromocyclododecane (HBCD), 2-bis(2,4,6-tribromophenoxy) ethane* (BTBPE); Hexabromobenzene (HBB); (2,3-Dibromopropyl) (2,4,6-tribromophenyl) ether* (DPTE); pentabromoethylbenzene* (PBEB) and 2,3,4,5,6-pentabromotoluene* (PBT). Σ_7PBDEs includes BDE-28, -47, -99, -100, -153, -154 and -183. Σ_{11}PBDEs includes BDE-28, -47, -99, -100, -153, -154, -183, -206, -207, -208 and -209.

Maternal blood was also analyzed for DL activity and PFASs[4]: perfluorohexane sulfonate* (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid* (PFNA), perfluorodecanoid acid* (PFDA), perfluoroundecanoic acid* (PDUdA), perfluorododecanoic acid* (PFDoA) and perfluorotridecanoic acid* (PFTrDA), perfluoro-n-hexanoic acid* (PFHxA), perfluoro-n-heptanoic acid* (PFHpA), perfluoro-n-tetradecanoic acid* (PFTeDA), potassium perfluorobutane sulfonate* (PFBS), perfluorooctanesulfonamide* (FOSA), metylperfluorooctanesulfonamide* (N-MeFOSA), metylperfluorooctanesulfonamidoethanol* (N-

[3] The BFRs marked with * are not included in the Stockholm Convention.
[4] The PFASs marked with * are not included in the Stockholm Convention.

MeFOSE), etylperfluorooctanesulfonamid* (N-EtFOSA) and etylperfluorooctanesulfonamidoethanol* (N-EtFOSE).

2.2.1 Analysis of OCPs, PCBs and BFRs

The analytical method is based on Brevik (Brevik 1978) with further modifications described in Müller et al. (2016), Müller et al. (2017) and in Polder et al. (2008). In brief, one to five grams of matrix were weighed in and added the internal standards: PCB-29, -112 and -207 (Ultra Scientific, N. Kingstown, RI, USA), 2-endo,3-exo,6-exo,8,9,10,10-heptachlorobornane (DE-TOX 409; LGC Standards GmbH, Wesel, Germany), BDE-77, -119, -181 and $^{13}C_{12}$BDE-209 (Cambridge Isotope Laboratories, Inc., MA, USA). Lipids were extracted twice with cyclohexane and acetone (3:2). The lipid percentage was determined gravimetrically. Lipid extracts were divided in two aliquots, which were treated with ≥ 97.5% H_2SO_4 (Fluka Analytical®) for analysis of OCPs group I, PCBs and BFRs or through a Gel Permeation Column (GPC) for analysis of OCPs group II.

Separation and detection of the OCPs group I and PCBs were performed on a HRGC using a dual capillary column system with dual electron capture detectors (Polder et al. 2008). Separation and detection of BFRs were performed on a HRGC–LRMS configured with a split/splitless injector (Agilent Technologies, Santa Clara, US). The BFRs were monitored using ECNI in SIM mode at m/z 79/81 for PBDEs, HBCD, BTBPE, PBEB and PBT, at m/z 79/551 for HBB and at m/z 160/79 for DPTE. Separation and detection of OCPs group II and CHBs were performed on a high resolution gas chromatograph (HRGC) coupled to a low resolution mass spectrometric (LRMS) detector operating in electron capture negative ionization (ECNI) in selected ion monitoring (SIM) mode.

2.2.2 Analysis of dioxin-like (DL) activity

DL activity in maternal blood was determined by Dioxin-Responsive Chemically Activated Luciferase eXpression (DR CALUX®) bioassay. The DR CALUX assay is based on a genetically modified H4IIE rat hepatoma cell line, which contains the firefly luciferase reporter gene under the transcriptional control of aryl hydrocarbon receptor (AhR). DL compounds bind to the AhR. The

analytical procedures were according to Biodetection Systems (BDS) protocols[5], with slight modifications. In brief, 1-2 grams of maternal blood were weighed in and extracted three times with hexane:diethyl ether solution (97:3). For lipid removal, the extracts were eluted through an acid silica column using hexane:diethyl ether solution (97:3). After evaporation, the samples were redissolved in dimethyl sulfoxide (DMSO). Cells were seeded in a 96 wells plate in 100 µL of alpha minimal essential medium (Gibco, Life Technologies AS, Norway) supplemented with 10% fetal calf serum (FCS) (VWR International, LLC Radnor, USA). Cells were exposed to 100 µL of sample extract, 2,3,7,8-TCDD calibration standards or DMSO controls dissolved in medium (0.4% DMSO). After 24-h exposure, cells were lysed and DL activity of the extracts was measured on a Centro XS^3 LB 960 luminometer (Berthold Technologies Gmbh, Germany), interpolated in the 2,3,7,8 TCDD calibration curve and expressed as picogram (pg) CALUX TEQ per gram of lipid and pg CALUX TEQ per gram of plasma.

2.2.3 Analysis of PFASs

The analytical procedure of PFASs is described by (Gronnestad et al. 2016). In brief, 1 mL of the maternal blood was weighed in Falcon centrifuge tubes (VWR International, LLC Radnor, USA) and added internal standards containing a ^{13}C-labeled PFAS mix (Wellington Laboratories). The samples were extracted twice with methanol (MeOH). For lipid removal, active carbon (EnviCarb) was used. Separation and detection of PFASs were performed using a high-performance liquid chromatography (HPLC) with a Discovery C18 column, connected to a C18 pre-column (Supelco, Sigma-Aldrich, Oslo, Norway) and a liquid chromatography (LC) tandem mass spectrometry (MS-MS) (API 3000, LC/MS/MS System).

2.2.4 QA/QC

For analyses of OCPs, PCBs and BFRs, the analytical batches included one non-spiked and two spiked samples of the same matrix as the analyzed (i.e. low fat cow milk, placenta from ewe, plasma from dog), three blanks (solvents), one harp seal blubber (*Pagophilus groendlandicus*) as internal reference material (IRM) for most POPs, and one minke whale blubber (*Balaenoptera acutorostrata*) as IRM for toxaphenes. For analyses of PFASs in maternal blood, the analytical

[5] www.bds.nl

series included one non-spiked (blind) and two spiked plasma samples from dog, three blanks (solvents) and three controls of serum (AMAP ring test).

For OCPs, PCBs, BFRs, PFASs, the lowest level of detection (LOD) for individual compounds was defined as three times the noise level for each compound. The LOD (ng/g wet weight (ww)) ranged for OCPs group I: 0.002-0.041, PCBs: 0.002-0.08, OCPs group II: 0.014-0.626, CHBs: 0.012-0.099, 7PBDEs: 0.003-0.367, BDE-206-209: 0.003-0.25, HBCD: 0.097-1.583, and PFASs: 0.02-0.27 (Table S1 and S2). The means of the relative recoveries ranged for OCPs group I: 77-159%, PCBs 100-127%, OCPs group II: 104-161%, 7PBDEs: 75-107%, BDE-206-209: 80-136%, HBCD: 99-116% and PFASs: 73-104%. If the relative recoveries were below 70% or above 130%, compounds were corrected for recoveries. Positive procedural blanks were found for some compounds. When the blank concentrations were consistent for all blank samples, results were corrected for blanks. The analytical quality was approved by satisfactory quality control measures, i.e. within the accreditation requirements, and results within the accepted ranges for the analysed Certified Reference Materials (CRM 2525, 350) and interlaboratory tests (AMAP: human serum).

For DL activity in maternal blood, the analytical batches included 2 blanks (solvents) per 10 samples, and two non-spiked and four spiked FCS (3 pM standard of TCDD) and one BDS reference material per 48 samples. The LOQ (pg/g CALUX TEQ ww) ranged between 0.15-1.29. Blank samples did not exceed the LOQ and recoveries ranged between 93-107%. With every DL activity measurement, an IRM (1.5 pM standard of TCDD) was included, and all IRMs and the BDS reference material fell within the accepted range.

2.3 Data treatment and statistics

Detection rate is defined as the percentage of samples with a detectable value, i.e. above LOD. The compounds with detection rate below 65% were only reported with range. Compounds with detection rate above 65% were reported with percentiles and range, and the actual determined concentrations for samples below LOD were used. Missing values were replaced with the lowest determined value of the compound. (Darnerud et al. 2015; Müller et al. 2016).

Spearman's rank correlations coefficient was used to examine the interrelationship of POPs in maternal blood and to assess the correlations of POPs between the maternal/infant compartments. Comparison of POP concentrations between compartments and correlations were calculated excluding values below LOD, and including only the compounds with at least 9 paired samples above LOD (Fisher et al. 2016; Needham et al. 2011; Vizcaino et al. 2014). Concentration ratios (ng/g lw) were calculated between paired samples for compounds with correlation coefficient above 0.7 (Needham et al. 2011):

$Ratio_{MB/BM}$ = MB/BM $Ratio_{MB/PL}$ = MB/PL

$Ratio_{CB/PL}$= CB/PL $Ratio_{CB/MB}$ =CB/MB $Ratio_{CB/BM}$ = CB/BM

where BM is the breast milk concentration, MB is the maternal blood concentration, PL is the placental concentration and CB is the cord blood concentration. Due to non-normal distribution, Wilcoxon matched-pairs signed rank test was used to determine significant difference in lipid percentage and POP concentrations between the compartments. In addition, a quantile regression model was run to assess whether the efficiency of transfer from maternal blood to cord blood and to breast milk varied across different maternal blood concentrations, i.e. if the regression coefficient varied between low and high concentrations of the predictor.

For compounds with correlation coefficients below 0.7, only observational comparison of the concentrations between compartments are given.

Stata/SE 14.0 for Windows (StataCorp, College Station, TX) were used for the calculations, and the differences were considered statistically significant when $p < 0.05$.

3. Results

3.1 Characteristics of the participants

Characteristics of the mothers and infants are presented in Table 1. All 48 mothers were healthy, primiparous, non-smokers and born in Tanzania. The majority of the mothers were within the normal BMI (18.5-24.9 kg/m^2), whereas one was underweight (below 18.5 kg/m^2), six were overweight (25.0-29.9 kg/m^2) and one was obese (above 30.0 kg/m^2). Occupation was separated

into two groups: farming and non-farming occupations. Income was divided into two groups: below 50 000 TZS (below 27 USD) and 51 000-500 000 TZS (27.5-270 USD) per month. All mothers had mixed diets, i.e. vegetables, meat and dairy products. Geophagy (eating clay soil/Pemba) during pregnancy was reported by all the 48 mothers.

Table 1: Characteristics of the mothers and infants.

	Number of observations*	**Mean**	**Min**	**Max**
Age	47	22	19	30
BMI (kg/m^2) before pregnancy	43	22.6	18.4	30.9
Weight gain during pregnancy (kg)	43	6.6	2	17
Gestational age (weeks)	47	38.5	36	40
Birth weight (kg)	47	3.2	2.5	4.1
Birth length (cm)	47	48.8	42	52
Head circumference (cm)	47	34.7	33	37
	Number of observations*	**Number**	**%**	
Infant gender (male/female)	47	25/22	53/47	
Farming as occupation	47	21	45	
Other occupation than farming	47	26	55	
Rural residence	47	22	47	
Urban residence	47	25	53	
Live near industrial activities	47	10	21	
Education (primary/ secondary school)	46	27/19	59/41	
Income (<50 000 /51 000-500 000 TZS**)	45	12/33	27/73	

**Due to missing information for some of the variables, the results are based on the number of observations given in the table. **100 000 TZS= 44.8 USD per 26.01.2017.*

3.2 Concentrations and profiles of POPs in maternal blood

The concentrations detected in maternal blood are presented in Table 2. p,p´-DDE dominated in 43 of 48 samples, while BDE-99 dominated in 3, HBCD in 1 and dioxin-like activity in 1 sample. p,p´-DDE contributed on average 94% to ΣDDTs. The ratio of p,p´-DDE/ p,p´-DDT ranged between 7.8 and 1013. For ΣDDTs, the maternal blood concentrations were above 100 ng/g lw in 60 % of the mothers, and above 1000 ng/g lw in 12.5%. BDE-47 contributed on average 79.6% to Σ_7PBDE. For Σ_7PBDEs, 8.3 % were above 100 ng/g lw.

HCB, γ-HCH, α-HCH, β-HCH, *cis*- chlordane, oxychlordane, *trans*-nonachlor, heptachlor, cis-heptachlor epoxide, trans-heptachlor epoxide, endosulfan sulfate, α-endosulfan, β-endosulfan, toxaphenes, mirex, technazen, quintozen and pentachloranillin, BDE-28, -206, -208, BTBPE, HBB, DPTE, PBEB and PBT were below LOD in all the maternal blood samples and are not further discussed.

The interrelationship between the POPs is presented in Table S3. PFASs correlated with each other (r= 0.43-0.86), and DL activity (pgTEQ/g lw) correlated to BDE-47 (r=0.36) and $\sum_7$PBDEs (r=0.33).

Table 2: Summary of POPs concentrations (ng/g lw*, **) in maternal blood from Arusha, Tanzania. Percentage of samples above LOD, median and range (min-max) are given in the Table. For compounds where less than 65% of the samples are above LOD, only range (min-max) is given.

N=48	% > LOD	Median	Min	Max		% > LOD	Median	Min	Max
Lipid %		0.55	0.38	0.97					
Dieldrin [b]	8		nd	164					
p,p'-DDE [a]	100	113	28.6	1890	BDE-28	nd			
p,p'-DDD [a]	90	6.61	nd	19.6	BDE-47[a]	65	1.96	nd	83.2
p,p'-DDT [b]	27		nd	91.8	BDE-99[b]	29		nd	167
∑DDTs		**117**	**29.9**	**1910**	BDE-100[b]	29		nd	27.6
PCB-153 [b]	29		nd	116	BDE-153[b]	10		nd	11.7
∑OCs		***146***	***29.9***	***1910***	BDE-154[b]	4		nd	9.65
PFOA[a*]	90	0.21	nd	1.13	BDE-183[b]	2		nd	3.88
PFNA[a*]	81	0.17	nd	0.45	BDE-206[b]	nd			
PFDA[a*]	85	0.14	nd	0.40	BDE-207[b]	6		nd	37.4
PFUdA[b*]	27		nd	0.17	BDE-208[b]	nd			
PFHxS[b*]	38		nd	0.73	BDE-209[b]	6		nd	51.2
PFOS[a*]	100	0.50	0.08	1.54	***∑7PBDEs***		***2.25***	**nd**	***299***
∑PFASs*		***1.18***	***0.18***	***3.14***	***∑11PBDEs***		***2.43***	**nd**	***299***
Dioxin-like activity**	92	30.2	nd	114	HBCD	2		nd	181

∑OCs= dieldrin, ∑DDTs, PCB-153

∑7PBDEs= BDE-28, -47, -99, -100, -153, -154, -183

∑11PBDEs= BDE-28, -47, -99, -100, -153, -154, -183, -206, -207, -208, -209

a= calculated with actual detected concentrations below LOD, zero values replaced with the lowest determined concentration above zero

b= calculated in the lower bound determination limit (nd= 0)

*= ng/mL plasma

**=pg CALUX TEQ/g lipid

Nd= not detected (below LOD)

Table 3: Summary of POPs concentrations (ng/g lw) in breast milk, placenta and cord blood from Arusha, Tanzania. Percentage of samples above LOD, median and range (min-max) are given in the table. For compounds where less than 65% of the samples are above LOD, only range (min-max) is given.

	Breast milk (N=47)				**Placenta (N= 45)**				**Cord blood (N=46)**			
	% > LOD	**Median**	**min**	**max**	**% > LOD**	**Median**	**min**	**max**	**% > LOD**	**Median**	**min**	**max**
Lipid%		1.3	0.23	14.0		0.78	0.13	1.40		0.13	0.05	0.37
HCB	85	1.41	nd	29.8	nd				nd			
α-HCH	17		nd	3.86	nd				nd			
β-HCH	32		nd	13.5	nd				nd			
γ-HCH	92	0.48	nd	7.42	nd				nd			
∑HCH		**0.90**	**nd**	**24.5**	**nd**				**nd**			
Oxychlordane	60		nd	12.9	nd				nd			
c-chlordane	19		nd	1.10	nd				nd			
t- nonachlor	19		nd	4.02	nd				nd			
∑CHL			**nd**	**12.9**	**nd**				**nd**			
Dieldrin	66	4.52	nd	205	na				9		nd	131
p,p'-DDE	100	135	28.8	2280	100	58.7	14.5	754	100	167	30.1	2300
p,p'-DDD	70	0.48	nd	23.7	nd				59		nd	30.3
p,p'-DDT	72	3.59	nd	251	11		nd	42.9	39		nd	79.4
∑DDTs		**135**	**29.9**	**2300**		**58.7**	**14.5**	**758**		**181**	**30.1**	**2320**
PCB-28	60		nd	15.9	nd				nd			
PCB-52	13		nd	2.15	nd				nd			
PCB-101	na				na				na			
PCB-138	92	1.80	nd	87.4	nd				nd			
PCB-153	57		nd	30.5	nd				37		nd	37.3
PCB-180	64		nd	18.0	nd				nd			
∑7PCBs		**4.45**	**nd**	**157**	**nd**						**nd**	**37.3**
BDE-28	nd				nd				nd			
BDE-47	92	5.30	nd	253	100	2.51	0.56	60.9	9		nd	91.6
BDE-99	92	8.23	nd	371	98	3.74	nd	45.6	9		nd	175
BDE-100	77	2.09	nd	106	42		nd	10.4	2		nd	33.7
BDE-153	66	1.44	nd	39.4	4		nd	3.83	nd			

BDE-154	2		nd	16.8	2		nd	0.90	nd			
BDE-183	11		nd	6.11	2		nd	7.92	4		nd	197
BDE-206	na				2		nd	12.5	nd			
BDE-207	na				nd				13		nd	235
BDE-208	na				nd				nd			
BDE-209	na				24		nd	324	4		nd	168
∑7PBDEs		**19.8**	**nd**	**786**		**7.35**	**1.17**	**117**			**nd**	**301**
∑PBDEs						**7.87**	**1.17**	**347**			**nd**	**433**
HBCD	2		nd	28.1	nd				17		nd	1280

∑7PBDEs= BDE-28, -47, -99, -100, -153, -154, -183

∑PBDEs= BDE-28, -47, -99, -100, -153, -154, -183, -206, -207, -208, -209

a= calculated with actual detected concentrations below LOD, zero values replaced with the lowest determined concentration above zero

b= calculated in the lower bound determination limit (nd= 0)

nd= not detected (below LOD)

na= not analyzed

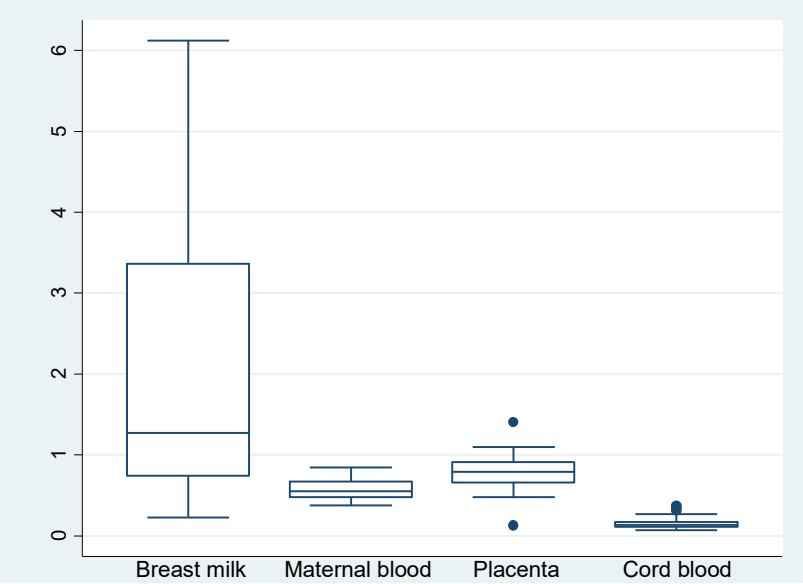

Fig. 1: *Lipid% of breast milk, maternal blood, placenta and cord blood. The boxplot presents the lipid% in breast milk, maternal blood, placenta and cord blood. The lipid% were significantly different (p=0.000) between the compartments. (The central band represents the median, the upper and lower end of the box represents the third and first quartiles, respectively, and whiskers are the highest/lowest 1.5 × interquartile range. Observations outside these intervals are drawn as outliers (circles)). The two maternal/infant couples with breast milk lipids of 9% and 14% are not included in the figure.*

3.2 Distribution of lipids and POPs between breast milk, maternal blood, placenta and cord blood

The POP concentrations detected in breast milk are reported in Müller et al. (2016) and Müller et al. (2017). Table 2 and 3 present the POP concentrations detected in the maternal/infant compartments. The lipid percentages were significantly different between the maternal/infant compartments ($p<0.001$) (*Fig*. 1). Lipid percentage was higher in breast milk than in maternal blood, placenta and cord blood in 68% of the paired samples, while placenta had higher lipid percentage than the other compartments in 33% of the paired samples. Cord blood had the lowest lipid percentage in all paired samples. Due to the differences in lipid percentages, lipid adjusted concentrations were used in the comparison of POPs between maternal and infant compartments.

p,p′-DDE, p,p′-DDD, p,p′-DDT, PCB-153, BDE-47, BDE-99 and BDE-100 were detected above LODs in a sufficient number of paired samples ($N>9$) to compare the concentrations between compartments. The compounds with correlation coefficients below 0.7 between compartments, and therefore only compared between the paired samples, are presented in Table 4.

p,p′-DDE was detected in 100% of the samples in all compartments and was strongly correlated between the compartments ($r=0.89$-0.98) (*Fig*. 2). Wilcoxon signed rank test showed that p,p′-DDE concentrations in maternal blood were significantly higher than in placenta ($p=0.0000$), but significantly lower than in cord blood ($p=0.0007$) and breast milk ($p=0.0001$) (*Fig*. S2). In addition, p,p′-DDE concentrations in cord blood were significantly higher than in placenta ($p=0.0000$), but not significantly different from breast milk ($p>0.1$). The ratios of p,p′-DDE concentrations between the compartments are presented in Table 5. The quantile regression model (Table 6, *Fig*. S3) showed a higher maternal blood regression coefficient for high (0.75-0.90) quantiles of cord blood and breast milk than for lower quantiles (0.25-0.50), suggesting an increased efficiency of p,p'-DDE transfer to cord blood and breast milk when maternal blood concentrations increase.

Heptachlor, cis-heptachlor epoxide, trans-heptachlor epoxide, α-endosulfan, β-endosulfan, toxaphenes, mirex, technazen, quintozen and pentachloranillin, BTBPE, HBB, DPTE, PBEB and PBT were not detected in any of the breast milk, placenta and cord blood samples.

Table 4: Comparison between lipid-adjusted concentrations breast milk, maternal blood, placenta and cord blood for compounds with spearman correlation coefficients (rho) < 0.7. Comparisons are performed for compounds detected in more than 9 paired samples. Only concentrations above LOD are included.

	Maternal blood vs Breast milk			Maternal blood vs Placenta			Cord blood vs Maternal blood			Cord blood vs Placenta			Cord blood vs Breast milk		
	N	rho	% MB>BM	N	rho	% MB>PL	N	rho	% CB>MB	N	rho	% CB>PL	N	rho	% CB>BM
p,p´-DDD	30	0.16	93	nc			24	0.46*	75	nc			19	-0.04	90
p,p´-DDT	13	0.68*	15	nc			11	0.49	64	nc			18	0.53*	28
PCB-153	11	0	82	nc			nc			nc			9	-0.05	100
BDE-47	27	-0.06	26	29	0.32	52	nc			nc			nc		
BDE-99	12	0.32	42	13	-0.04	46	nc			nc			nc		
BDE-100	13	-0.12	62	nc			nc			nc			nc		

N= the number of paired samples included in the comparison

rho= spearman correlation coefficients

%= percentage of N which is higher in the indicated compartment.

nc= not compared between compartments due to detected concentrations in an insufficient number of paired samples (N<9).

*p-value<0.05

Table 5: Ratios of p,p´-DDE concentrations (lw) between breast milk (BM), maternal blood (MB), placenta (PL) and cord blood (CB).

	N	Mean	Median	Min	Max
MB/BM	47	0,95	0,76	0,55	7,32
MB/PL	45	2,20	2,19	1,03	3,96
CB/MB	46	1,34	1,28	0,62	2,66
CB/PL	44	2,87	2,76	1,34	5,54
CB/BM	45	1,28	1,01	0,54	9,25

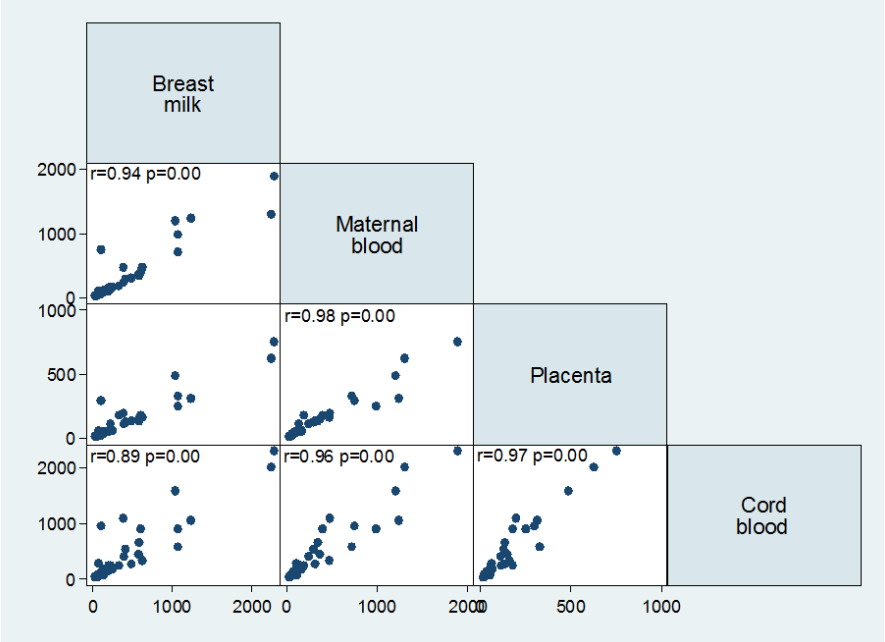

Fig. 2: *Correlations of lipid-adjusted p,p´-DDE concentrations between breast milk, maternal blood, placenta and cord blood. Spearman correlation coefficients (r) and p-values (p) are indicated in the windows.*

Table 6: Quantile regression analyses of *p,p´-DDE* transfer from maternal blood to cord blood and breast milk (lipid-adjusted concentrations).

	Quantile	r2	Coefficient	95% CI	*p-value*
CB/MB					
	25	0.5891	0.905	0.792-1.02	*0.0000*
	50	0.7021	1.214	1.067-1.360	*0.0000*
	75	0.7625	1.532	1.279-1.785	*0.0000*
	90	0.779	1.898	1.642-2.155	*0.0000*
BM/MB					
	25	0.5266	0.986	0.718-1.254	*0.0000*
	50	0.6974	1.202	1.092-1.312	*0.0000*
	75	0.8029	1.510	1.384-1.636	*0.0000*
	90	0.8744	1.723	1.660-1.787	*0.0000*

4. Discussion

4.1 Assessment of prenatal POPs exposure

This is the first study to report PFASs and DL activity in humans from Tanzania. Maternal blood was used to assess prenatal POPs exposure due to larger sample amounts than for the other compartments.

This study revealed that the Tanzanian infants are exposed to a wide range of POPs, both in terms of concentrations and compounds, during prenatal life. p,p´-DDE was the most frequently detected POP (100%) in maternal blood with a median concentration within the same range as non-malaria areas in South Africa (Channa et al. 2012), slightly higher than reported in blood from pregnant women in USA (Woodruff et al. 2011), and twofold higher than maternal blood in Canada (Fisher et al. 2016) and Norway (Caspersen et al. 2016). The ratios of DDE/DDT in this study did not reflect recent use of DDT by the Tanzanian mothers, hence the source of exposure is mainly related to DDT residues from previous use for malaria vector control and agricultural activities.

BDE-47 was the most frequently detected PBDE (65%), but BDE-99 was detected in highest concentrations (range <LOD-167 ng/g lw). Noteworthy, 8% of the Tanzanian maternal blood samples were above the 75th percentile of BDE-47 reported in maternal blood in a Californian mother-child cohort, which are known to be among the highest PBDE exposed populations in the world (Sagiv et al. 2015). PBDEs are not manufactured in Tanzania and imported electronic devices, textiles and furniture may be sources of PBDEs, especially from improper waste disposals (Muller et al. 2016).

Dieldrin was detected in 4 maternal blood samples ranging from LOD to 164 ng/g lw. However, dieldrin was detected in 65% of breast milk samples from the same mothers, and the intake of dieldrin exceeded the tolerable daily intake for 6% of the nursing infants (Müller et al. 2017). The higher detection rate in breast milk compared to maternal blood may be related to the lower lipid content in maternal blood and higher LODs, suggesting breast milk as a better matrix for monitoring dieldrin exposure. The present study urge the identification of dieldrin sources in Tanzania as these sources may pose human health risks.

The DL activity detected in the present study was within the same range as detected in industrialized, European countries (Long et al. 2006). The positive correlations between concentrations of DL activity and PBDEs (BDE-47 r=0.36 and $\sum_7$PBDEs r=0.33), and not PCB-153, may suggest that brominated rather than chlorinated dioxins and furans contribute to the dioxin-like activity (Ericson Jogsten et al. 2010). Although the constituents of the DL activity may vary between individuals and countries, the chlorinated and brominated counterparts are toxicologically equal, and thus, represent similar health risks (van den Berg et al. 2013). The general population in European countries is mainly exposed to dioxins through the diet (EFSA 2012). In Tanzania, the population may have additional exposure to DL compounds from other sources, like open fire cooking, burning of municipal waste (Polder et al. 2016) and geophagy (Müller et al. 2016 and 2017). In contrast to the European population, the exposure to DL compounds in the Tanzanian population may be reduced considerably if proper public information and governmental management are implemented.

PCB-153 was the only PCB detected in the maternal blood samples. However, low concentrations of PCB-138 and PCB-180 were also detected in breast milk from the same mothers. Since global bans and restrictions in the 1970s, PCBs in the global environment and humans are declining. Nevertheless, the existing PCB concentrations are still posing human health concerns considering their high toxic potency. Müller et al. (2017) estimated daily intakes of nondioxin-like PCBs, including PCB-153, in breast milk from the same participants as in the present study, and the intake concentrations exceeded a health based guidance value of 10 ng/kg body weight/day (AFSSA 2007; VKM 2008) in 50% of the studied nursing infants.

In accordance with other studies, PFOS was the most abundant of the PFASs detected in maternal blood, followed by PFOA, PFDA and PFNA (Lau et al. 2007). However, the concentrations of PFASs in this study were lower than detected in other countries (Inoue et al. 2004; Kishi et al. 2015; Okada et al. 2013; Starling et al. 2014; Stein et al. 2012). In African countries, exposure pathways of PFASs may be different from the western world, due to lower living standards, less access to modern consumer products and lower housing quality (Hanssen et al. 2010). Due to the rapid development, industrialization and modernization in Tanzania, it is likely that the importation of products containing PFASs will increase in the coming years.

4.2 Distribution of POPs between breast milk, maternal blood, placenta and cord blood

In this study, breast milk and placenta showed higher detection rates of OCs and BFRs than maternal and cord blood (Table 2 and 3). The majority of compounds were below LOD in cord blood, which are likely related to the low sample amounts and low lipid percentage of cord blood.

In general, POPs are transferred by passive diffusion between maternal/infant compartments depending on their lipid percentages. Thus, the lipid adjusted POP concentrations between compartments are expected to correlate and the ratios should be close to one. p,p′-DDE was the only compound that was strongly correlated between all compartments (r=0.89-0.98). The other POPs, i.e. p,p′-DDD, p,p′-DDT, PCB-153, BDE-47, BDE-99 and BDE-100, showed lower detection rates, lower concentrations and weaker correlations between compartments ($r<0.7$) than p,p′-DDE. Therefore, the distributions of other POPs than p,p′-DDE are assessed with caution.

Strong correlations of p,p′-DDE concentrations between maternal blood and cord blood are also found by others (Vizcaino et al. 2014; Waliszewski et al. 2000). The correlations of p,p′-DDE concentrations between cord blood, placenta and maternal blood (r=0.96-0.98), indicate a substantial placental transfer. In addition, the significantly lower concentrations of p,p′-DDE in placenta compared to maternal blood (p=0.000) and cord blood (p=0.000) indicate that p,p′-DDE are not retained in placenta, but rather cross the barrier and enter the fetal circulation. The median MB/PL and CB/PL ratio showed that p,p′-DDE concentrations in maternal blood were 2 times higher than in placenta, and 2.8 times higher in cord blood than in placenta, respectively. Furthermore, p,p′-DDE concentrations were significantly higher in cord blood than in maternal blood (p=0.001), with median CB/MB ratio of 1.3. Higher concentrations of POPs in cord blood compared to maternal blood are reported in some studies (Vizcaino et al. 2014, Fisher et al. 2016; Jeong et al. 2018), while others report contradictory results (Dewan et al. 2013; Needham et al. 2011). Considering the constant fetal need and flow of lipids from the mother to the fetus during pregnancy (Herrera et al. 2006), p,p′-DDE may build up and equilibrate between the fetal compartments independently from the equilibrium that is established between the maternal compartments (Vizcaino et al. 2014; Alcorn and McNamara, 2003). Likewise, lower efficiency of

fetal detoxification processes and lower excretion capacity than the mother, may be contributing factors to the higher concentrations of p,p′-DDE in cord blood (Waliszewski et al. 2001).

p,p′-DDE concentrations were also significantly correlated between maternal blood and breast milk (r=0.94). The significantly lower concentrations of p,p′-DDE in maternal blood than in breast milk (p=0.018) is contrary to what Dewan et al. (2013) reported. This may be explained by the large variation of POP concentrations in breast milk collected during the first 3 weeks of lactation (WHO 2009). Moreover, OCs are found to associate to triglycerides in milk fat (Hugunin and Bradley 1971). Due to de novo synthesis of fatty acids by the mammary glands (Ortiz-Olaya et al. 1996), breast milk may contain a different composition of lipids than maternal blood, and thus, may explain the differences in p,p′-DDE concentrations between maternal blood and breast milk. Different composition of lipids may also explain the differences between the other compartments. However, since the fatty acids in the samples were not specified in the present study, this hypothesis could not be addressed.

In accordance with other studies (Dewan et al. 2013; Vizcaino et al. 2014), the distribution of p,p′-DDE between the maternal/infant compartments indicated that lipophilic compounds are transferred through placenta and to breast milk with mechanisms other than just passive diffusion (Table 5). Although less robust data for the PCB-153 and PBDEs, their distributions correspond with this hypothesis (Table 4). Additionally, the present study found that the transfer rate of p,p`-DDE from maternal blood to cord blood and to breast milk increased when maternal blood concentrations increased. These findings concur with maternal-infant transfer of drugs (Blackburn 2017), but has not previously been reported for chemicals. Differences in degree and positioning of halogenation, molecular weight and hydrophobicity are reported to influence the POPs transfer through membranes, such as the placenta (Midasch et al. 2007; Hanssen et al. 2010; Frederiksen et al. 2010; Tsukimori et al. 2013; Jeong et al. 2018). The placenta and mammary glands regulate the transfer of exogenous and endogenous substances to cord blood and breast milk, respectively, through interactions between transport proteins (Young et al. 2003; Myllynen et al. 2009; Needham et al. 2011). POPs, like p,p′-DDE, may disturb these interactions, which may lead to increased influx of p,p′-DDE, and also other POPs, from maternal blood to cord blood and to breast milk. POPs may also have other toxic effects on the mammary and placental functions, like vascular

lesions and degeneration of endothelial cells, resulting in higher permeability, which ease the penetration of POPs and other toxic compounds (Backlin et al. 1998). Whether such mechanisms explain the increased efficiency of maternal transfer with increasing maternal POP concentrations observed in this study need further research.

The best matrices for assessing prenatal and postnatal exposure appear to be cord blood and breast milk, respectively. The higher p,p′-DDE concentrations in cord blood and breast milk compared to maternal blood (median CB/MB ratio 1.3 and MB/BM ratio 0.8) found in this study, suggest that POP concentrations in maternal blood may underestimate the actual concentrations in the fetus and the nursing infant. The insignificant difference in p,p′-DDE concentrations between cord blood and breast milk ($p>0.1$) and the median CB/BM ratio of 1, demonstrate that breast milk collected close to delivery may serve as a more suitable indicator of the cord blood concentrations than maternal blood, and thereby, more appropriate for prenatal exposure assessment. This is also suggested for PBDEs by others (Kim et al. 2012). Furthermore, Hernik et al. (2016) found insignificant differences in concentrations of PCB-101, BDE-47 and BDE-99 between breast milk and cord blood concentrations. However, contradictory to the p,p′-DDE distribution in the present study, Hernik et al. (2016) found significantly higher concentrations in breast milk than cord blood for p,p'-DDE and other POPs, i.e. HCB, HCHs, PCBs (-138, -153, -170, -180) and BDE-153. The inconsistency between studies may be explained by individual variations and low number of participants in the studies. Noteworthy, cord blood and breast milk close to delivery are of great value for the infant and collection of these matrices must be of restricted volumes. Due to its non-invasive nature, placenta has been suggested to serve as a good prenatal exposure assessment tool (Jeong et al. 2018). However, the placenta concentration was significantly lower than the cord blood concentration in this study, suggesting that the placenta concentration may underestimate prenatal exposure. With ethical considerations taken into account, further studies with a higher number of participants, larger sample amounts and specified fatty acid profiles are urged to increase the knowledge of POPs distribution between mothers and infants during perinatal life.

4.3 Strengths and limitations

Breast milk, maternal blood, cord blood and placenta were collected close to delivery and analyzed at the same laboratory using the same chemical method, which give strengths to the comparison of POPs between maternal/infant compartments.

Sensitive and specific high-resolution GC high-resolution MS (HRGC-HRMS) techniques are used to quantify dioxins (reported as WHO-TEQ). However, these methods are time-consuming, expensive and require large sample volumes. DR CALUX® was a suitable method for this project, both in terms of low sample amounts and low cost per sample. DR CALUX® does not quantify specific compounds, but measures the collective response of the AhR agonists/antagonists and thereby expresses the total dioxin-like activity of chemical mixtures, also accounting for possible interactions (synergism, addition and/or antagonism) between chemicals (reported as CALUX-TEQ). Despite important methodological differences, strong correlations between CALUX-TEQ and WHO-TEQ are reported from human studies (Koppen et al. 2001; Van Wouwe et al. 2004). In studies with higher CALUX-TEQ than WHO-TEQ for chlorinated dioxins, brominated dioxins and furans are likely to account for the deviation, especially in humans with high concentrations of PBDEs (Ericson Jogsten et al. 2010). Due to the low sample amounts, confirmation of the CALUX-TEQ with HRGC-HRMS analysis for chlorinated and/or brominated dioxins and furans was not possible in the present study.

5. Conclusion

For the first time, BFRs, PFASs and dioxin-like compounds are reported in human blood samples from Tanzania. This study adds baseline information on the presence of PFASs in Tanzania, and contribute to the scarce knowledge of PFASs in African countries. In addition, this study reveals that chemicals, like BFRs and PFASs, produced in Western countries and imported to Tanzania through consumer products, accumulate in pregnant women and subsequently their infants during fetal life. The detected POP concentrations indicate a substantial prenatal exposure, especially to DDTs, PBDEs and dioxin-like compounds, which may lead to adverse developmental health effects.

The distribution of p,p´-DDE between maternal/infant compartments did not correspond to only passive diffusion according to the compartments` lipid percentages. Moreover, this study indicated

that p,p′-DDE concentrations detected in maternal blood may lead to underestimation of the concentrations in cord blood and breast milk. Breast milk collected close to delivery was shown to be a more suitable indicator of the p,p′-DDE concentrations in cord blood than maternal blood. Although WHO recommends collection of breast milk after 3 weeks for purposes like monitoring, body burden estimation and risk assessments for nursing infants, breast milk collected close to delivery may be more suitable for prenatal exposure assessments. Altogether, these results suggest that the prenatal exposure to POPs in the Tanzanian infants may be even higher than assessed from maternal blood concentrations in this study. Further studies are needed to assess adverse health effects related to chemical exposure in infants and children in Tanzania.

Acknowledgements

We sincerely thank the participating mothers, nurses, doctors and laboratory technicians at Mount Meru Regional Referral Hospital. We thank Dr. Kasumuni, Mrs Lyaruu and Mr Karengi for their effort in sampling, preparation and storage of samples. Dr. Morten Sandvik, Hanne Moberg Larsen and Ellen Hage are also thanked for the technical support in Norway. Biodetection Systems, especially Emiel Felzel and Dr. Peter Behnisch, are acknowledged for the license and technical support for the DR CALUX® analysis. The here used DR CALUX® cells are property from BioDetection Systems. This study received financial support from the Research Council of Norway (NFR nr: 204051).

Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

References

AFSSA. 2007. Opinion of the French Food Safety Agency on the establishment of relevant maximum concentrations for nondioxin-like polychlorobiphenyls (NDL-PCBs) in some foodstuffs (2006-sa-0305). Maisons, Alfort, France: French Food Safety Agency. Available at: https://www.anses.fr/fr/system/files/RCCP2006sa0305EN.pdf.

Alcorn J, McNamara PJ. 2003. Pharmacokinetics in the newborn. Adv Drug Deliv Rev; 55: 667–86.

Asante KA, Adu-Kumi S, Nakahiro K, Takahashi S, Isobe T, Sudaryanto A, et al. 2011. Human exposure to PCBs, PBDEs and HBCDs in Ghana: Temporal variation, sources of exposure and estimation of daily intakes by infants. Environ Int 37:921-928.

Backlin BM, Persson E, Jones CJ, Dantzer V. 1998. Polychlorinated biphenyl (PCB) exposure produces placental vascular and trophoblastic lesions in the mink (mustela vison): A light and electron microscopic study. APMIS 106:785-799.

Barr DB, Wang RY, Needham LL. 2005. Biologic monitoring of exposure to environmental chemicals throughout the life stages: Requirements and issues for consideration for the national children's study. Environ Health Perspect 113:1083-1091.

Blackburn S. 2017. Maternal, Fetal, & Neonatal Physiology - E-Book: A Clinical Perspective, Edition 5. Elsevier Health Sciences. eBook ISBN: 9780323473958 Hardcover ISBN: 9780323569118.

Brevik EM. 1978. Gas chromatograhic method for the determination of organochlorine pesticides in human milk. Bull Environ Contam Toxicol 19:281-286.

Caspersen IH, Kvalem HE, Haugen M, Brantsæter AL, Meltzer HM, Alexander J, et al. 2016. Determinants of plasma PCB, brominated flame retardants, and organochlorine pesticides in pregnant women and 3 year old children in the Norwegian mother and child cohort study. Environ Res 146:136-144.

Channa K, Rollin HB, Nost TH, Odland JO, Sandanger TM. 2012. Prenatal exposure to DDT in malaria endemic region following indoor residual spraying and in non-malaria coastal regions of South Africa. Sci Total Environ 429:183-190.

Chen X, Chen JS, Zhang L, Li JG, Yao L, Self SG, et al. 2015. Concentrations of PCDDs, PCDGs and dl-PCBs in the blood of childbearing-aged women living in the vicinity of a chemical plant in Tianjin: A primary study. Chemosphere 118:1-4.

Cohn BA, Wolff MS, Cirillo PM, Sholtz RI. 2007. DDT and breast cancer in young women: New data on the significance of age at exposure. Environ Health Perspect 115:1406-1414.

Cooper GS, Martin SA, Longnecker MP, Sandler DP, Germolec DR. 2004. Associations between plasma DDE concentrations and immunologic measures in African-American farmers in North Carolina. Environ Health Perspect 112:1080-1084.

Dalvie MA, Myers JE, Lou Thompson M, Dyer S, Robins TG, Omar S, et al. 2004. The hormonal effects of long-term DDT exposure on malaria vector-control workers in Limpopo Province, South Africa. Environ Res 96:9-19.

Darnerud PO, Atuma S, Aune M, Bjerselius R, Glynn A, Grawe KP, et al. 2006. Dietary intake estimations of organohalogen contaminants (dioxins, PCB, PBDE and chlorinated pesticides, e.g. DDT) based on Swedish market basket data. Food Chem Toxicol 44:1597-1606.

Darnerud PO, Lignell S, Aune M, Isaksson M, Cantillana T, Redeby J, et al. 2015. Time trends of polybrominated diphenylether (PBDE) congeners in serum of Swedish mothers and comparisons to breast milk data. Environ Res 138:352-360.

Dewan P, Jain V, Gupta P, Banerjee BD. 2013. Organochlorine pesticide residues in maternal blood, cord blood, placenta, and breastmilk and their relation to birth size. Chemosphere 90:1704-1710.

EFSA. 2007. Opinion of the scientific panel on plant protection products and their residues on a request from the commission on the risks associated with an increase of the MRL for dieldrin on courgettes. EFSA Journal:1-48.

EFSA. 2012. European food safety authority: Update of the monitoring of concentrations of dioxins and PCBs in food and feed. EFSA Journal 10:2832.

Ericson Jogsten I, Hagberg J, Lindstrom G, Bavel B. 2010. Analysis of POPs in human samples reveal a contribution of brominated dioxin of up to 15% of the total dioxin TEQ. Chemosphere 78:113-120.

Eriksson U, Karrman A. 2015. World-wide indoor exposure to polyfluoroalkyl phosphate esters (PAPs) and other PFASs in household dust. Environ Sci Technol 49:14503-14511.

Eskenazi B, Marks AR, Bradman A, Fenster L, Johnson C, Barr DB, et al. 2006. In utero exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment among young Mexican American children. Pediatrics 118:233-241.

Fisher M, Arbuckle TE, Liang CL, LeBlanc A, Gaudreau E, Foster WG, et al. 2016. Concentrations of persistent organic pollutants in maternal and cord blood from the maternal-infant research on environmental chemicals (MIREC) cohort study. Environ Health 15:59.

Frederiksen M, Thomsen C, Froshaug M, Vorkamp K, Thomsen M, Becher G, et al. 2010. Polybrominated diphenyl ethers in paired samples of maternal and umbilical cord blood plasma and associations with house dust in a danish cohort. Int J Hyg Environ Health 213:233-242.

Gronnestad R, Villanger GD, Polder A, Kovacs KM, Lydersen C, Jenssen BM, et al. 2016. Maternal transfer of perfluoroalkyl substances in hooded seals. Environ Toxicol Chem / SETAC.

Hanssen L, Rollin H, Odland JO, Moe MK, Sandanger TM. 2010. Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: Results of a pilot study. J Environ Monit 12:1355-1361.

Hernik A, Strucinski P, Buckley BT, Goralczyk K, Czaja K, Korcz W, et al. 2016. Relationship between paired cord blood and milk POPs concentrations as a tool for assessing perinatal exposure, a pilot study. Human and Ecological Risk Assessment: An International Journal 22:1456-1468.

Herrera E, Amusquivar E, Lopez-Soldado I, Ortega H. 2006. Maternal lipid metabolism and placental lipid transfer. Horm Res 65 Suppl 3:59-64.

Hugunin AG, Bradley RL, Jr. 1971. Distribution of organochlorine pesticides among some milk components. J Dairy Sci 54:355-359.

Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, et al. 2004. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: Assessment of PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect 112:1204-1207.

Jakobsson K, Fang J, Athanasiadou M, Rignell-Hydbom A, Bergman A. 2012. Polybrominated diphenyl ethers in maternal serum, umbilical cord serum, colostrum and mature breast milk. Insights from a pilot study and the literature. Environ Int 47:121-130.

Jeong Y, Lee S, Kim S, Park J, Kim HJ, et al. 2018. Placental transfer of persistent organic pollutants and feasibility using the placenta as a non-invasive biomonitoring matrix. Sci Total Environ. 15;612:1498-1505.

Kannan K, Tanabe S, Giesy JP, Tatsukawa R. 1997. Organochlorine pesticides and polychlorinated biphenyls in foodstuffs from Asian and Oceanic countries. Rev Environ Contam Toxicol 152:1-55.

Kaseva M, Mbuligwe S, Kassenga G. 2002. Recycling inorganic domestic solid wastes: Results from a pilot study in Dar es Salaam city, Tanzania. Resources, Conservation and Recycling 35: 243-257.

Kim TH, Bang du Y, Lim HJ, Won AJ, Ahn MY, Patra N, et al. 2012. Comparisons of polybrominated diphenyl ethers concentrations in paired South Korean cord blood, maternal blood, and breast milk samples. Chemosphere 87:97-104.

Kishi R, Nakajima T, Goudarzi H, Kobayashi S, Sasaki S, Okada E, et al. 2015. The association of prenatal exposure to perfluorinated chemicals with maternal essential and long-chain polyunsaturated fatty acids during pregnancy and the birth weight of their offspring: The Hokkaido study. Environ Health Perspect 123:1038-1045.

Koppen G, Covaci A, Van Cleuvenbergen R, Schepens P, Winneke G, Nelen V, et al. 2001. Comparison of CALUX-TEQ values with PCB and PCDD/F measurements in human serum of the Flanders environmental and health study (FLEHS). Toxicol Lett 123:59-67.

Langer P. 2010. The impacts of organochlorines and other persistent pollutants on thyroid and metabolic health. Front Neuroendocrinol 31:497-518.

Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. 2007. Perfluoroalkyl acids: A review of monitoring and toxicological findings. Toxicol Sci 99:366-394.

Long M, Andersen BS, Lindh CH, Hagmar L, Giwercman A, Manicardi GC, et al. 2006. Dioxin-like activities in serum across European and Inuit populations. Environ Health 5:14.

Lorber M. 2008. Exposure of Americans to polybrominated diphenyl ethers. J Expo Sci Environ Epidemiol 18:2-19.

Midasch O, Drexler H, Hart N, Beckmann MW, Angerer J. 2007. Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: A pilot study. International archives of occupational and environmental health 80:643-648.

Müller MH, Polder A, Brynildsrud OB, Karimi M, Lie E, Manyilizu WB, et al. 2017. Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in human breast milk and associated health risks to nursing infants in Northern Tanzania. Environ Res 154: 425–434.

Müller MH, Polder A, Brynildsrud OB, Lie E, Loken KB, Manyilizu WB, et al. 2016. Brominated flame retardants (BFRs) in breast milk and associated health risks to nursing infants in Northern Tanzania. Environ Int 89-90:38-47.

Myllynen P, Immonen E, Kummu M, Vahakangas K. 2009. Developmental expression of drug metabolizing enzymes and transporter proteins in human placenta and fetal tissues. Expert Opin Drug Metab Toxicol 5:1483-1499.

Needham LL, Grandjean P, Heinzow B, Jorgensen PJ, Nielsen F, Patterson DG, Jr., et al. 2011. Partition of environmental chemicals between maternal and fetal blood and tissues. Environ Sci Technol 45:1121-1126.

Ntow WJ, Tagoe LM, Drechsel P, Kelderman P, Gijzen HJ, Nyarko E. 2008. Accumulation of persistent organochlorine contaminants in milk and serum of farmers from Ghana. Environ Res 106:17-26.

Okada E, Kashino I, Matsuura H, Sasaki S, Miyashita C, Yamamoto J, et al. 2013. Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003-2011. Environ Int 60:89-96.

Ortiz-Olaya N, Flores ME, DeSantiago S. 1996. Significance of lipid consumption during lactation. Revista de investigacion clinica; organo del Hospital de Enfermedades de la Nutricion 48:473-478.

Polder A, Gabrielsen GW, Odland JO, Savinova TN, Tkachev A, Loken KB, et al. 2008. Spatial and temporal changes of chlorinated pesticides, PCBs, dioxins (PCDDs/PCDFs) and brominated flame retardants in human breast milk from Northern Russia. Sci Total Environ 391:41-54.

Polder A, Savinova TN, Tkachev A, Loken KB, Odland JO, Skaare JU. 2010. Concentrations and patterns of persistent organic pollutants (POPs) in selected food items from Northwest Russia (1998-2002) and implications for dietary exposure. Sci Total Environ 408:5352-5361.

Polder A, Muller MB, Brynildsrud OB, de Boer J, Hamers T, Kamstra JH, et al. 2016. Dioxins, PCBs, chlorinated pesticides and brominated flame retardants in free-range chicken eggs from peri-urban areas in Arusha, Tanzania: Concentrations and implications for human health. Sci Total Environ 551-552:656-667.

Prouillac C, Lecoeur S. 2010. The role of the placenta in fetal exposure to xenobiotics: Importance of membrane transporters and human models for transfer studies. Drug Metab Dispos 38:1623-1635.

Sagiv SK, Kogut K, Gaspar FW, Gunier RB, Harley KG, Parra K, et al. 2015. Prenatal and childhood polybrominated diphenyl ether (PBDE) exposure and attention and executive function at 9-12 years of age. Neurotoxicol Teratol 52:151-161.

Skaare JU, Tuveng JM, Sande HA. 1988. Organochlorine pesticides and polychlorinated biphenyls in maternal adipose tissue, blood, milk, and cord blood from mothers and their infants living in Norway. Arch Environ Contam Toxicol 17:55-63.

Starling AP, Engel SM, Whitworth KW, Richardson DB, Stuebe AM, Daniels JL, et al. 2014. Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian mother and child cohort study. Environ Int 62:104-112.

Stein CR, Wolff MS, Calafat AM, Kato K, Engel SM. 2012. Comparison of polyfluoroalkyl compound concentrations in maternal serum and amniotic fluid: A pilot study. Reprod Toxicol 34:312-316.

The Stockholm Convention. 2016. Available at: http://chm.pops.int/.

Tsukimori K, Morokuma S, Hori T, Takahashi K, Hirata T, Otera Y, et al. 2013. Characterization of placental transfer of polychlorinated dibenzo-p-dioxins, dibenzofurans and polychlorinated biphenyls in normal pregnancy. The journal of obstetrics and gynaecology research 39:83-90.

UNEP. 2009. Global monitoring report unep/pop/cop.4/33. Available at: http://chm.pops.int/TheConvention/ConferenceoftheParties/Meetings/COP4/COP4Documents/tabid/531/Default.aspx.

Van den Berg M, Denison MS, Birnbaum LS, Devito MJ, Fiedler H, Falandysz J, et al. 2013. Polybrominated dibenzo-p-dioxins, dibenzofurans, and biphenyls: Inclusion in the toxicity equivalency factor concept for dioxin-like compounds. Toxicol Sci 133:197-208.

Van Dyk JC, Bouwman H, Barnhoorn IE, Bornman MS. 2010. DDT contamination from indoor residual spraying for malaria control. Sci Total Environ 408:2745-2752.

Van Wouwe N, Windal I, Vanderperren H, Eppe G, Xhrouet C, Massart AC, et al. 2004. Validation of the CALUX bioassay for PCDD/F analyses in human blood plasma and comparison with GC-HRMS. Talanta 63:1157-1167.

Vizcaino E, Grimalt JO, Fernandez-Somoano A, Tardon A. 2014. Transport of persistent organic pollutants across the human placenta. Environ Int 65:107-115.

VKM. 2008. Risk assessment of nondioxin-like PCBs in Norwegian food. Opinion of the panel on contaminants of the Norwegian Scientific Committee for Food Safety Oslo, Norway. Available at: http://www.vkm.no/dav/e1bd5f7615.pdf.

Waliszewski SM, Aguirre AA, Infanzon RM, Siliceo J. 2000. Carry-over of persistent organochlorine pesticides through placenta to fetus. Salud publica de Mexico 42:384-390.

Waliszewski SM, Aguirre AA, Infanzon RM, Silva CS, Siliceo J. 2001. Organochlorine pesticide concentrations in maternal adipose tissue, maternal blood serum, umbilical blood serum, and milk from inhabitants of Veracruz, Mexico. Arch Environ Contam Toxicol 40:432-438.

WHO. 2007. Fourth WHO-coordinated survey of human milk for persistent organic pollutants in cooperation with UNEP guidelines for developing a national protocol. Available at: http://www.who.int/foodsafety/chem/popprotocol.pdf.

WHO. 2009. Biomonitoring of human milk for persistent organic pollutants (POPs). Available at: http://www.who.int/foodsafety/chem/pops_biomonitoring/en/.

WHO. 2010. Persistent organic pollutants: Impact on child health. Available at: http://apps.who.int/iris/bitstream/10665/44525/1/9789241501101_eng.pdf.

Woodruff TJ, Zota AR, Schwartz JM. 2011. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. Environ Health Perspect 119:878-885.

Young AM, Allen CE, Audus KL. 2003. Efflux transporters of the human placenta. Adv Drug Deliv Rev 55:125-132.