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Intra- and intercompartmental associations between levels of organochlorines in maternal plasma, cord plasma and breast milk, and lead and cadmium in whole blood, for indigenous peoples of Chukotka, Russia†‡

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Long-range transport of pollutants towards circumpolar regions emphasizes the need for up-to-date and reliable biological monitoring data. This paper explores the use, reliability and availability of maternal blood (MB) and plasma (MP), cord blood (CB) and plasma (CP) and mother's milk (MM) in terms of assessing exposure to persistent toxic substances (PTSs). It is concluded that MP has the best combination of availability, sensitivity in terms of number of PTSs, their detection frequency and concentrations, and physiological relevance. The study group consisted of 48 pregnant women of indigenous origin from the Chuchki district in the eastern Russian arctic. Blood, CB and MM specimens were collected from all women and MP, CP and MM were analyzed for the Arctic Monitoring and Assessment Programme (AMAP) suite of organochlorines (OCs) and metals (Pb and Cd in MB and CB). Generally speaking, the levels of PTSs coincided with those indicated in several AMAP publications from Chukotka and other areas of northern Russia. The correlations of PTS concentrations between the three body fluid compartments exceeded the minimum statistical requirements of $\alpha = 0.05$ and $\beta = 0.20$ for most of the compounds, with $r > 0.46$ except for Cd ($r = 0.05$); lipid adjustments for the OCs did not affect the r-values to any significant extent. The majority of the inter-OC correlations within compartments also fulfilled the indicated statistical condition. Careful consideration is given to the replacement of concentrations below the detection limit, OC detection frequency, the criteria for log-transformation of the data, analytical uncertainty, and biological variability. Practical implications of the findings are explored.

Introduction

In 1997–1998, the Arctic Monitoring and Assessment Programme (AMAP) presented the first report on the state of pollution in the arctic.[1] This report clearly documented that persistent toxic substances (PTSs) accumulated in arctic food chains. However, the impact this has had on the Russian arctic indigenous peoples needed to be clarified. The exposures to PTSs, and thus the potential ill effects associated with them, are especially critical for indigenous peoples in the arctic. This is generally because of long-range transport of pollutants towards the north,[2] but especially the consumption of traditional diets, that are typically often extensively contaminated,[3] which in turn can result in high PTS body burdens.

Humans are thought to be particularly vulnerable to exposure from these pollutants *in utero* and during the first months of life through breast-feeding.[4–6] Although some results, conclusions and recommendations related to human health in the arctic were presented in a recent AMAP report,[7] clarification was needed about sampling and interpretations of levels in different body fluids. There are three main tissues in which the levels of OCs are most often measured in order to assess the exposure to the unborn child and neonate, namely MP, CP and MM (and for metals whole blood rather than plasma).

While correlations between the levels of contaminants in these compartments have been reported,[8–10] there is some dispute as to which medium is the most suitable for these assessments. Further, measuring contaminants in all three body fluids is not always economically viable. The specific objective of the present study is to help clarify unresolved issues related to specimen collection, sampling, laboratory analysis and approaches employed in data analysis,

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with special emphasis on detection limits, detection frequencies and lipid adjustments of PTS concentrations.

Materials and methods

Geography and study population

The Chukchi Autonomous Okrug (CAO) is located in the far northeast corner of Russia and is separated from Alaska by the Bering Strait. The CAO occupies an area of 737 700 square kilometres and includes the regions of Anadyr, Chaunski and Chukchi, as well as several islands such as Wrangel Island. About 50% of the territory is located above the Arctic Circle. The total population of the CAO is 50 700 (March 1, 2005); in 1989 the population was about 164 000.[11] The capital is Anadyr and it has a population of 11 028. About 60 nationalities are represented in the CAO including 16 native groups of the north (Chukchi, Eskimo (Yupics), Evens, Koryaks, Chauvans, Yukagirs, Tungueses, Nenetses, Lamuts and others). Russians comprise 66% of the population, indigenous peoples 20% and Ukrainians 9%.[11] The ethnic breakdown for the native peoples is 50% Chukchi, 4.5% Eskimo (Yupics), 4% Evens and 3% Chauvans, among others.

From October 2001 to March 2002, pregnant indigenous women who had been admitted to a local delivery department in the CAO were invited to participate in the study. The participating delivery departments in the CAO were: Anadyr City delivery department of the Central Okrug Hospital, from May 2001 until November 2001; Settlement of Ugolnye Kopi (Anadyrsky district) delivery department of the District Hospital, from April 2001 until March 2002; Settlement of Lavrentya (Chukotski district, north-eastern Chukotka) delivery department of the District Hospital, from August 2001 until March 2002. The delivering women were from the 15 settlements shown in Fig. 1.

Sampling

Blood samples from the mother were extracted from the ulnar vein on day 1 to day 3 after delivery. Blood from the umbilical cord was taken immediately after ligation and separation, regardless of whether or not the placenta had been released.

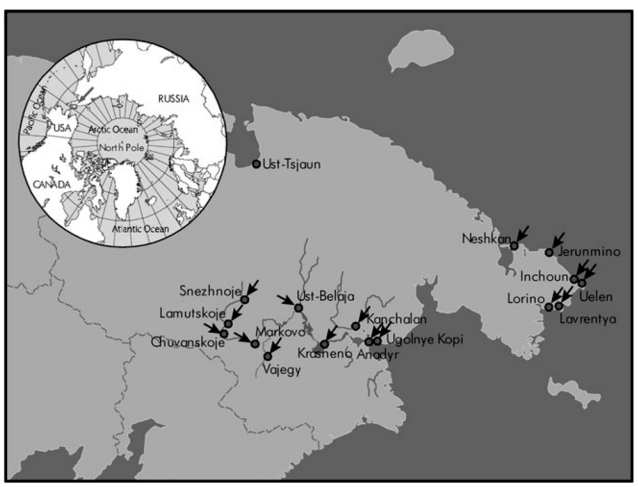

Fig. 1 Chukotka Autonomous Okrug.

All blood collection was performed using Becton Dickinson Vacutainers (USA), with different vials for organochlorines (BD366457, K_3EDTA) and metals (BD367863, K_2EDTA). Most of the blood specimens were subsequently centrifuged at 3000 rpm before transferring the plasma by pipette (3.5 mL, No 86.1172.001, Sarstedt, Germany) into special pre-cleaned vials (7 mL clear vial with screw cap, Supelco, USA, for OCs; and whole blood into SC flat-base tubes No 60.542.024 Sarstedt, Germany for the metals). Milk samples (30 mL) were all collected during the 3rd week after delivery (days 14–21) by gently squeezing the milk directly into polycarbonate screw-top containers EN 829 (Sarstedt, Germany). Blood and milk samples were kept frozen at −20 °C and transported in thermo-containers to the Center for Environmental Chemistry (CEC), Scientific Production Association (SPA) "Typhoon", Federal Service of the Russian Federation for Hydrometerology and Environmental Monitoring, Obninsk, Russia. All samples of milk, whole blood or plasma analyzed had volumes between 2 and 6 mL.

Analyses of PTSs in plasma and milk samples

Sample treatment and analyses. The 48 samples were processed and analysed by the CEC laboratory.

The levels of the following PTSs were determined: α-HCH, β-HCH, γ-HCH, *p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT, *o,p′*-DDE, *o,p′*-DDD, *o,p′*-DDT; ToxP -26,-50,-62, heptachlor, *cis*-chlordane, *trans*-chlordane, *oxy*-chlordane, dieldrin, mirex, HCB and PCB congener numbers: 28/31, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187.

Plasma and milk samples were thawed at room temperature. Aliquots (2 to 6 mL of the specimens) were weighed to the nearest 0.01g and were diluted with an equivalent volume of methanol. Subsequently the OCs were extracted with hexane : methyl-*tert*-butyl ether (1 : 1), the extract was dried over anhydrous sodium sulfate, and lipids were removed using gel permeation chromatography (20 grams of Bio-Bead SX-3 packed in glass columns equipped with Teflon caps with hexane : DCM (1 : 1), as the eluant). The extracts were further cleaned and fractionated with a 3% deactivated silica gel column, a modification of the method by Pauwels *et al.*[12] Batches of the plasma/milk samples were analyzed for PTSs; each batch included 12 plasma or milk samples, a procedural blank, a spiked matrix sample, and a solution of isotopically-labeled standards (PCB congeners, EC-4058 Cambridge Isotope Laboratory) as part of the quality assurance and the quality control (QA–QC) programs. The content of lipids was determined gravimetrically in an aliquot of the extract (20% of the extracted volume). All analyses were carried out by gas chromatography–mass spectrometry (GC–MS) employing a Varian Saturn 2200 T instrument (Varian Inc., CA, USA).

QA–QC criteria and quality control. Recovery of analytes was monitored using ^{13}C-labelled analogues added to all samples. Recovery rates were between 50 and 120% for all the internal standards. The procedural blank acceptance criteria were: 0.1 ng (OC pesticides) and 1 ng (PCB congeners). These values were derived from the analyses of 433 samples (36 batches). The performances of the individual analytical

instruments were established on a daily basis and it included checks for instrument sensitivity using standard calibrants, as well as standard chromatographic and mass resolution criteria.

The CEC participated in three rounds of inter-laboratory comparisons, namely the AMAP Ring Tests,[13] and achieved the stated objective of being within 20% of the assigned concentrations.

Analyses of whole blood for toxic metals

Sample treatment and analyses. Metal analyses were done in the same facility as the OCs using standard procedures for mercury (Hg), lead (Pb) and cadmium (Cd). Briefly, the blood samples were thawed at room temperature and 0.8 mL was transferred to a 5 mL vial and mixed with 0.8 mL of 0.1% triton X-100 (Union Carbide Chemicals and Plastics Co., Inc.) and 4 mL of 2 N nitric acid (Ultra Pure Product). The solution was centrifuged for 15 min at 3000 rpm and the supernatant was transferred to polyethylene cups for quantification. In the case of Pb and Cd, each analytical batch included 10–12 samples of whole blood, a procedural blank, a field blank, a sample of a standard certified reference material (IRMM BCR 195, whole blood), as well as a duplicate sample. In all, 40 sample batches were analyzed. Cd and Pb concentrations were measured by electrothermal atomic-absorption spectrometry using a Perkin Elmer model Z3030 spectrophotometer with Zeeman background correction and using pyro-coated graphite tubes with L'vov platform. Standard additions and ammonium pyrophosphate as matrix modifier (2000 mg L^{-1}) were employed. Quantification of Hg was accomplished by the standard "cold vapor" technique,[14] using potassium permanganate as the oxidizing agent and hydroxylamine chloride as the reducing agent. Each batch involved 10 samples of whole blood, a procedural blank, a field blank and a control sample.

Statistical procedures. Detection limits (DL) for PCBs, DDTs, HCB and the metals were calculated from the procedural blanks using the formula: $DL = t_{(n-1,\alpha=0.05)}SD$, where n is the number of measured blanks, t is the Student coefficient at a confidence level of 95%, and SD is the standard deviation. For the remaining OCs, analyses of control samples spiked with low levels (close to the DL) of the specific compounds were used to calculate the DL. For the 2–6 mL aliquots analyzed, the DL increased with decreasing volume, and the DL for 2 mL was used for both the OCs and metals, as the use of a sample-specific DL is impractical. For the sum of congeners, the DL was calculated employing the following formula: $\sum DL \text{ of congeners} = \sqrt{((DL_1^2 + DL_2^2 + \ldots DL_k^2)/k - 1)}$. Because 7 of the lipid concentrations in the cord plasma (CP) were marginally lower than the corresponding lipid-DL, we elected to replace them with the DL (rather than $DL/\sqrt{2}$) prior to the lipid normalization of the OCs.

In a preliminary survey of the distributions of the contaminant concentrations, it was clear that they were not normally distributed and for the descriptive statistics, the geometric mean was reported along with the range, arithmetic mean and its SD. All statistical manipulations were done with log-transformed [natural logarithm ($\log_e x$)] values. In case of the OCs, lipid adjustments were made after replacing concentrations below the DL with $DL/\sqrt{2}$. If more than 20% of the OC or metal concentrations were below the DL, data sets were not used in the statistical analyses, descriptive or otherwise. The latter restriction was not applied to the sum of PCBs, aroclor or sums of other OC pesticides.

The variation in the data was assessed before and after lipid adjustment using the coefficient of variation (V). Intercompartmental and intracompartmental correlation analyses were completed using linear regression to find the Pearson's regression coefficient. Power estimates employed the Fisher's z transformation for the critical value r as described by Zar.[15] All statistical procedures were carried out using the SPSS for Windows statistical package (version 14.0; SPSS Inc., Chicago, IL, USA).

Table 1 Demographics of the study population (n = 48)

		Frequency (N)	Percent
Age (mean 24.2)	Age groups		
	Under 21 years	17	35.4
	21–24	12	25
	25–29	10	20.8
	30 years and over	9	18.8
Ethnicity	Chukchi	39	81.3
	Eskimo (Yupics)	1	2.1
	Korjak	1	2.1
	Chuvanets	7	14.6
	Total	48	100
Residence of participants (Fig. 1)	Anadyr	6	12.5
	Kanchalan	2	4.2
	Chuvanskoje	2	4.2
	Snezhnoje	2	4.2
	Ust-Belaja	4	8.3
	Krasneno	1	2.1
	Vajegy	2	4.2
	Markovo	2	4.2
	Lamutskoje	2	4.2
	Uelen	2	4.2
	Lavrentya	3	6.3
	Lorino	6	12.5
	Neshkan	5	10.4
	Inchoun	4	8.3
	Jenurmino	5	10.4
	Total	48	100
Civil status	Married	33	68.7
	Single	15	31.3
	Total	48	100
Education	Part-time secondary school (< 10 years)	9	18.8
	Secondary school (10 years)	33	68.8
	Vocational education (> 10 years)	5	10.4
	Higher education (institute)	1	2.1
	Total	48	100
Parity (including this one)	1	20	41.7
	2	16	33.3
	3	5	10.4
	4	4	8.3
	5	1	2.1
	6	2	4.2
	Total	48	100

Table 2 Detection limits (μg L^{-1}) for all OCs and metals. The detection limits displayed in this table are for 2 mL samples

Name of substance	DL	Name of substance	DL
PCB-28/31 [CL3]	0.040	*cis*- Chlordane	0.010
PCB-52 [CL4]	0.040	∑Chlordanes	0.012
PCB-99 [CL5]	0.040	*o*,*p*′-DDE	0.010
PCB-101 [CL5]	0.060	*p*,*p*′-DDE	0.010
PCB-105 [CL5]	0.090	*o*,*p*′-DDD	0.010
PCB-118 [CL5]	0.140	*p*,*p*′-DDD	0.020
PCB-128 [CL6]	0.020	*o*,*p*′-DDT	0.080
PCB-138 [CL6]	0.070	*p*,*p*′-DDT	0.080
PCB-153 [CL6]	0.050	∑DDTs	0.023
PCB-156 [CL6]	0.020	Hexachlorobenzene	0.022
PCB-170 [CL7]	0.020	Dieldrin	0.040
PCB-180 [CL7]	0.030	Mirex	0.020
PCB-183 [CL7]	0.010	*trans*-Norachlor	0.010
PCB-187 [CL7]	0.010	*cis*-Norachlor	0.010
∑PCBs	0.020	ToxP-26	0.016
Aroclor 1260	0.200	ToxP-50	0.008
α-HCH	0.020	ToxP-62	0.030
β-HCH	0.020	∑ToxPs	0.018
γ-HCH	0.020	Cd	0.020
∑HCHs	0.017	Pb	5.000
oxy-Chlordane	0.020	Hg	0.700
trans-Chlordane	0.010	Lipids[a]	0.067

[a] DL for lipids is displayed in %.

Results

Population characteristics

Population characteristics are presented in Table 1. The average age of the women was 24.2 years and 35% were under 21 years of age; 68.8% had finished secondary education, 31.3% were single, and 41.7% were primiparous.

Descriptive statistics

The observed DL values are listed in Table 2, and the descriptive statistics for the PTSs and chlorinated pesticides are presented in Tables 3–5. The percentage of non-detects in breast milk exceeded 20% for PCBs-52, 101, 105, 128, γ-HCH, *trans*-chlordane, *o*,*p*′-DDE, *o*,*p*′-DDD, *o*,*p*′-DDT, dieldrin and ToxP-62, and thus are not reported in Table 3 (but see Table S1 in the ESI‡). By comparison, for MP the following are added to this list: PCB-28/31, α-HCH, the remaining chlordanes, *p*,*p*′-DDD, mirex, and ToxP-26 (see Table 4). Perusal of Table 5 shows that only 7 of the 43 OCs had a detection frequency (DF) over 80% in CP. In terms of the metals, Cd and Pb were present above the DL in all MB and CB samples. Hg was detectable in 68% of the samples, this is linked to its high DL, and thus the Hg results could not be examined further. None of the metals were determined in breast milk. The ratios of the arithmetic means of *p*,*p*′-DDE to *p*,*p*′-DDT in the three compartments were: 8.9 (MM), 9.8 (MP) and 8.9 (CP). Of the PCB congeners, 153 was the most abundant in both MM and MP. Congeners 99, 118, 138 and 153 account for 72% of the sum of all the PCBs tested in MM and 69% in MP. From the data in Tables 3 and 4, we can see that the average concentrations of the individual substances after fat-adjustment are higher in MP than in MM (except *trans*-nonachlor and the ToxPs). The average total lipid content in MM was 3.1% and in MP 0.54%. Because a biphasic distribution of the lipid concentrations in CP was observed (*cf.*, Fig. S1, S2 and S3 in the ESI‡), lipid adjustments were only made for discussion purposes (see Table 5). In MP, the magnitude of the lipid range was far narrower than in the two other compartments. The coefficient of variation [*V*; 100(SD/mean)] for the total lipids was 0.36 in MM, 0.22 in MP and 1.12 in CP.

Table 3 Descriptive statistics for milk (μg L^{-1} or μg kg^{-1} lipids)

A. Descriptive statistics for milk, not adjusted for fat content/μg L^{-1}

Name of congener	% missing	Min.	Max.	AM	SD	GM
PCB-28/31	2.1	DL	0.45	0.19	0.10	0.16
PCB-99	0.0	0.09	4.38	1.20	1.24	0.65
PCB-118	4.2	DL	4.67	1.27	1.20	0.75
PCB-138	0.0	0.12	4.10	1.07	1.03	0.67
PCB-153	0.0	0.29	34.44	4.99	6.46	2.31
PCB-156	6.3	DL	3.70	0.37	0.55	0.21
PCB-170	0.0	0.06	2.26	0.49	0.54	0.29
PCB-180	0.0	0.09	3.76	0.82	0.87	0.47
PCB-183	2.1	DL	0.55	0.15	0.14	0.09
PCB-187	2.1	DL	1.42	0.37	0.35	0.22
∑PCBs	0.0	1.02	46.81	11.50	11.84	6.59
α-HCH	8.3	DL	0.56	0.17	0.12	0.13
β-HCH	0.0	0.12	36.94	8.99	8.25	5.63
∑HCHs	0.0	0.19	37.58	9.19	8.37	5.85
oxy-Chlord.	2.1	DL	33.81	4.81	7.00	1.16
∑Chlord.	2.1	DL	34.74	5.03	7.27	1.21
p,*p*′-DDE	0.0	1.64	17.70	7.49	3.87	6.37
p,*p*′-DDD	0.0	0.02	1.00	0.26	0.22	0.17
p,*p*′-DDT	0.0	0.11	2.40	0.84	0.57	0.66
∑DDTs	0.0	1.97	19.85	8.70	4.39	7.44
HCB	0.0	0.48	25.69	5.82	5.17	3.96
Mirex	18.8	DL	1.95	0.28	0.41	0.10
trans-Non.	0.0	0.12	12.97	2.92	3.52	1.20
cis-Non.	0.0	0.02	1.75	0.36	0.46	0.17
ToxP-26	4.2	DL	2.59	0.48	0.64	0.19
ToxP-50	2.1	DL	3.45	0.54	0.69	0.25
∑ToxPs	2.1	DL	6.14	1.02	1.32	0.44

B. Descriptive statistics for milk adjusted for fat content/μg kg^{-1} lipids

Name of congener		**Min.**	**Max.**	**AM**	**SD**	**GM**
PCB-28/31	2.1	1	34	7	5	6
PCB-99	0.0	4	159	39	39	23
PCB-118	4.2	5	148	41	37	27
PCB-138	0.0	5	135	36	35	24
PCB-153	0.0	15	1252	167	225	82
PCB-156	6.3	>0.5	67	11	12	7
PCB-170	0.0	2	82	17	19	10
PCB-180	0.0	4	122	28	30	17
PCB-183	2.1	1	17	5	5	3
PCB-187	2.1	>0.5	48	12	12	8
∑PCBs	0.0	50	1702	384	396	233
α-HCH	8.3	1.00	25	6	5	5
β-HCH	0.0	6	1598	316	316	199
∑HCHs	0.0	10	1628	323	320	207
oxy-Chlord.	2.1	2	1070	163	246	41
∑Chlord.	2.1	1	1099	170	256	43
p,*p*′-DDE	0.0	62	812	266	161	225
p,*p*′-DDD	0.0	1	33	9	7	6
p,*p*′-DDT	0.0	3	160	31	29	23
∑DDTs	0.0	74	934	309	188	263
HCB	0.0	28	934	192	179	140
Mirex	18.8	>0.5	62	9	14	4
trans-Non.	0.0	6	595	100	133	43
cis-Non.	0.0	1	80	12	16	6
ToxP-26	4.2	>0.5	101	15	21	7
ToxP-50	2.1	>0.5	112	18	22	9
∑ToxPs	2.1	>0.5	213	33	43	16

Table 4 Descriptive statistics for maternal blood/plasma (μg L^{-1} or μg kg^{-1} lipids)

A. Descriptive statistics for maternal blood/plasma not adjusted for fat content/μg L^{-1}

Name of congener	% missing	Min.	Max.	AM	SD	GM
PCB-99	10.4	DL	0.79	0.26	0.23	0.15
PCB-118	20.0	DL	1.30	0.32	0.25	0.25
PCB-138	12.5	DL	0.92	0.28	0.23	0.19
PCB-153	0.0	0.08	4.08	1.03	1.10	0.50
PCB-156	8.3	DL	0.24	0.07	0.05	0.05
PCB-170	4.2	DL	0.61	0.14	0.13	0.09
PCB-180	8.3	DL	0.91	0.22	0.22	0.13
PCB-183	6.3	DL	0.14	0.04	0.03	0.03
PCB-187	0.0	0.01	0.40	0.09	0.09	0.06
∑PCBs	0.0	0.33	9.18	2.74	2.30	1.89
Aroclor	0.0	0.70	26.00	6.77	6.78	3.74
β-HCH	0.0	0.14	7.60	1.62	1.55	1.08
∑HCHs	0.0	0.14	7.60	1.63	1.55	1.09
p,p′-DDE	0.0	0.36	4.63	1.96	1.19	1.61
p,p′-DDT	16.7	DL	0.59	0.20	0.14	0.16
ΣDDTs	0.0	0.42	5.33	2.20	1.29	1.83
HCB	0.0	0.14	6.04	1.22	1.13	0.86
trans-Non.	0.0	0.01	1.76	0.42	0.51	0.16
ToxP-50	12.5	DL	0.29	0.07	0.08	0.03
∑ToxPs	18.8	DL	0.64	0.14	0.17	0.06
Cd	0.0	0.15	4.73	1.21	0.98	0.95
Pb	0.0	17	227	50	33	44

B. Descriptive statistics for maternal plasma adjusted for fat content/μg kg^{-1} lipids

PCB-99	10.4	4	207	52	51	30
PCB-118	20.0	13	233	62	47	49
PCB-138	12.5	6	188	55	47	38
PCB-153	0.0	15	870	207	237	97
PCB-156	8.3	2	43	13	10	10
PCB-170	4.2	3	136	27	28	17
PCB-180	8.3	4	203	43	47	25
PCB-183	6.3	1	28	8	7	6
PCB-187	0.0	3	76	18	18	11
∑PCBs	0.0	55	1873	547	491	362
Aroclor	0.0	101	5306	1361	1463	720
β-HCH	0.0	22	1949	328	362	207
∑HCHs	0.0	22	1949	330	362	208
p,p′-DDE	0.0	59	1029	381	236	308
p,p′-DDT	16.7	9	126	40	29	31
ΣDDTs	0.0	69	1185	428	255	351
HCB	0.0	29	1162	239	235	165
trans-Non.	0.0	3	382	81	100	30
ToxP-50	12.5	1	74	13	17	6
∑ToxPs	18.8	2	164	27	35	12

Table 5 Descriptive statistics for cord blood/plasma (μg L^{-1} or μg kg^{-1} lipids)

A. Descriptive statistics for cord blood/plasma not adjusted for fat content/μg L^{-1}

Name of congener	% missing	Min.	Max.	AM	SD	GM
∑PCBs	0.0	0.08	11.53	1.47	2.29	0.65
Aroclor	18.8	DL	33.43	3.64	6.56	1.04
β-HCH	8.3	DL	7.96	0.80	1.40	0.29
∑HCHs	8.3	DL	8.11	0.80	1.42	0.29
p,p′-DDE	0.0	0.11	5.01	0.89	1.08	0.56
∑DDTs	0.0	0.14	5.54	0.99	1.19	0.64
HCB	0.0	0.01	3.96	0.69	0.83	0.37
Cd	0.0	0.05	3.97	0.43	0.72	0.21
Pb	0.0	14	210	45	30	39

B. Descriptive statistics for cord plasma adjusted for fat content/μg kg^{-1} lipids

∑PCBs	0.0	63	3262	753	625	549
Aroclor	18.8	100	10 771	1649	1976	873
β-HCH	8.3	10	1529	412	359	243
∑HCHs	8.3	9	1529	413	361	240
p,p′-DDE	0.0	138	1700	567	347	476
∑DDTs	0.0	140	1883	630	369	536
HCB	0.0	13	1950	439	377	312

[a] Table 5B is only displayed in order to show the fact that these adjustments are invalid compared to the adjusted OC values in Tables 3 and 4.

The mean concentration of Pb was 1.1 times higher in maternal blood than in cord blood and the mean concentration of Cd was 2.8 times higher in maternal blood.

Intercompartmental correlations

Both the unadjusted *r*-values (italic font) and the lipid-adjusted *r*-values (normal font) are summarized in Table 6. The unadjusted PCB correlations between the compartments exceeded 0.65, except for congener 118 and 156 which had values of 0.47 and 0.55 (MM–MP), respectively. For the OC pesticides and Pb, all the *r*-values were above 0.67, but with $r = 0.05$ for Cd. It is clear that the lipid adjusted *r*-values were of comparable magnitude. The variation in the data (*V*) also changed with lipid adjustments for selected congeners in the compartments; the *V* decreased by about two-fold in CP, but increased somewhat in MP and MM.

Typical normal scatter plots are presented in Fig. S4 (PCB-99 in MM and MP), S5 (PCB-118 in MM and MP), S6 and S7 (PCB-156 in MM and MP, before and after lipid adjustment, respectively), and S8 (Cd in MB and CB) in the ESI‡.

Intracompartmental correlations

The *r*-values for all compounds within each respective compartment using the lipid unadjusted values are available in the ESI‡ (Tables S2–S4). For milk (Table S2‡), all the PCBs were highly inter-correlated with one another as well as with the OCs, with a lack of correlations with *p,p′*-DDT and *p,p′*-DDE as exceptions. PCB congeners 28/31 and 156 exhibited somewhat lower correlations with other PCBs and OC pesticides. *p,p′*-DDE was correlated with all the other compounds with *r*-values between 0.34 and 0.78, while *p,p′*-DDD and *p,p′*-DDT were not ($r < 0.25$). Exceptions to the latter were the correlations between *p,p′*-DDD or *p,p′*-DDT with α- or β-HCH (*r*-range 0.31–0.56). In MP, correlations among the PCBs ranged 0.58–1.0 and between PCBs and the other OCs 0.44–0.93, except *p,p′*-DDT for which the range was 0.01–0.22. PCB congener 118 generally had the lowest *r*-values. The unique relationships between *p,p′*-DDT and the other OCs seen in MM is again observed here. The correlations between metals in whole blood and other compounds in plasma had $r < 0.35$. However, the *r*-value between Cd and Pb was 0.50. Although for CP there were fewer linear regressions, the general trends were similar to those seen in other compartments. Interestingly, there was no positive correlation between Pb and Cd in CP.

Table 6 Intercompartment correlations among PTSs (lipid adjusted data in normal font; unadjusted in italic font)

PCB-99	Maternal plasma	Milk
Maternal plasma	1.00	0.90 (0.77–1.00)
Milk	*0.90 (0.76–1.00)*	1.00
PCB-118	Maternal plasma	Milk
Maternal plasma	1.00	0.53 (0.27–0.78)
Milk	*0.47 (0.20–0.73)*	1.00
PCB-138	Maternal plasma	Milk
Maternal plasma	1.00	0.79 (0.61–0.97)
Milk	*0.82 (0.65–0.99)*	1.00
PCB-153	Maternal plasma	Milk
Maternal plasma	1.00	0.90 (0.76–1.00)
Milk	*0.92 (0.80–1.00)*	1.00
PCB-156	Maternal plasma	Milk
Maternal plasma	1.00	0.46 (0.20–0.73)
Milk	*0.55 (0.30–0.79)*	1.00
PCB-170	Maternal plasma	Milk
Maternal plasma	1.00	*0.74 (0.54–0.94)*
Milk	*0.83 (0.67–1.00)*	1.00
PCB-180	Maternal plasma	Milk
Maternal plasma	1.00	0.81 (0.64–0.99)
Milk	*0.89 (0.750–1.00)*	1.00
PCB-183	Maternal plasma	Milk
Maternal plasma	1.00	0.79 (0.61–0.97)
Milk	*0.79 (0.59–0.94)*	1.00
PCB-187	Maternal plasma	Milk
Maternal plasma	1.00	0.81 (0.64–0.99)
Milk	*0.85 (0.69–1.00)*	1.00
∑PCBs	Maternal plasma	Milk
Maternal plasma	1.00	0.85 (0.69–1.00)
Milk	*0.87 (0.72–1.00)*	1.00
Cord plasma	*0.69 (0.48–0.91)*	*0.74 (0.54–0.94)*
Aroclor 1260	Maternal plasma	
Maternal plasma	1.00	
Cord plasma	*0.78 (0.59–0.96)*	
***β*-HCH**	Maternal plasma	Milk
Maternal plasma	1.00	0.88 (0.73–1.00)
Milk	*0.90 (0.77–1.00)*	1.00
Cord plasma	*0.84 (0.67–1.00)*	*0.71 (0.50–0.92)*
∑HCHs	Maternal plasma	Milk
Maternal plasma	1.00	0.88 (0.74–1.00)
Milk	*0.91 (0.79–1.00)*	1.00
Cord plasma	*0.83 (0.67–1.00)*	*0.71 (0.51–0.92)*
***p,p′*-DDE**	Maternal plasma	Milk
Maternal plasma	1.00	0.71 (0.51–0.92)
Milk	*0.79 (0.61–0.97)*	1.00
Cord plasma	*0.78 (0.59–0.96)*	*0.70 (0.49–0.91)*
***p,p′*-DDT**	Maternal plasma	Milk
Maternal plasma	1.00	0.65 (0.42–0.88)
Milk	*0.72 (0.51–0.92)*	1.00
∑DDTs	Maternal plasma	Milk
Maternal plasma	1.00	0.73 (0.53–0.93)
Milk	*0.80 (0.62–0.98)*	1.00
Cord plasma	*0.76 (0.56–0.95)*	*0.67 (0.45–0.89)*
HCB	Maternal plasma	Milk
Maternal plasma	1.00	0.89 (0.75–1.00)
Milk	*0.86 (0.70–1.00)*	1.00
Cord plasma	*0.69 (0.47–0.90)*	*0.77 (0.58–0.96)*
***trans*-Nonachlor**	Maternal plasma	Milk
Maternal plasma	1.00	0.89 (0.75–1.00)
Milk	*0.90 (0.76–1.00)*	1.00
ToxP-50	Maternal plasma	Milk
Maternal plasma	1.00	0.86 (0.71–1.00)
Milk	*0.83 (0.66–1.00)*	1.00
∑ToxPs	Maternal plasma	Milk
Maternal plasma	1.00	0.91 (0.79–1.00)
Milk	*0.89 (0.75–1.00)*	1.00
Cd	Maternal blood	
Maternal blood	1.00	
Cord blood	*0.05 (−0.24–0.35)*	
Pb	Maternal blood	
Maternal blood	1.00	
Cord blood	*0.89 (0.76–1.00)*	

Distribution and power

Visual inspection of the plots showed that all data sets were skewed to the right before log transformation (*cf.* Fig. S9 and S10‡), with *p,p′*-DDE and the sum of DDTs in MM being exceptions (*e.g.*, Fig. S11 and S12‡). In this instance, we performed a comparative intercorrelation analysis (as in Table 6) for the sum of DDTs against *p,p′*-DDE to see if the *r*-values depended on transformation. The *r*-value changed from 0.993 [using log-transformed (log e_x values)] to 0.990 (without log transformation). Further, we also checked how the *r*-value behaved when a correlation analysis was performed between the sum of DDTs in MM (which perhaps did not need log transformation) and the sum of DDTs in MP (which needed log transformation), yielding *r*-values of 0.802 (both log-transformed) and 0.694 (both raw data). The study's sample size, $n = 48$, might be considered limited, although our calculations showed that at the 95% level of confidence the power $(1 - \beta)$ exceeds 0.8 as long as the *r*-value is above 0.4.

Discussion

Statistical analyses

Since 2 mL was the lowest volume of any sample in the analytical phase, the DLs were based on it. This conservative decision likely resulted in slight overestimations of the reported average concentrations. The statistical implications of this were not evaluated.

There are basically 3 criteria that will help in deciding whether or not a log-transformation of data is necessary;[16] namely skewness, the presence of outliers and unequal variation. There were some outliers in our dataset and certainly unequal variation occurred within each body-fluid compartment, especially for CP. All the OCs and metal concentrations were lognormally distributed except for the two DDTs in MM. However, for the latter the criteria of unequal variation and outliers were met. Lognormal distributions are also confirmed by the fact that the geometric means in Tables 3–5 are generally smaller than the arithmetic means. Even though some "upper-bound" outliers mainly related to age, parity and diet might be expected,[17] we saw no *a priori* reason to remove any. (Detailed examination of exposure risk factors was not an objective of the current paper.)

In the recent literature, one can find a wide range of detection frequencies adopted for use in descriptive and/or-statistical methods, (between 20% and 90%).[18,19] We opted for 80% as the minimum DF. There are several recommended approaches for dealing with the observations below the DL: (i) keep numbers below the DL; (ii) replace the numbers with 0; (iii) remove them; (iv) replace with DL/2 (or DL/$\sqrt{2}$); or (v), replace the observations with random numbers ("fill-in numbers").[20,21] All these methods potentially introduce bias, are dependent on the number of replacements, but also on the statistical procedures employed. We refrained from using zero because this would give too low estimates in the descriptive section. Since all the data needed to be log-transformed, we did not want to lose these low measurements. We adopted the DL/$\sqrt{2}$ approach since the skewness generally met the criteria set out for this choice by Hornung and Reed,[22] namely around

3 times the geometric standard deviation (GSD). In our unadjusted data set, the GSD was below 3 for 76% of the data in MP, 66% of the data in MM and 44% in CP. Because of this, and other reasons (see below), we felt that CP concentrations of OCs in our study should, formally speaking, not be lipid adjusted.

Variation in the data needs to be addressed at three different levels: (i) the variation in the raw-data; (ii) the difference in variation before and after lipid adjustment; and (iii) the variation in lipid distribution between the three compartments. These points will be addressed later in the discussion.

Descriptive statistics

Although all the women who participated in this study were native peoples, they are not representative of the diverse ethnic groups of Chukotka. However, since the main aim of this study was to describe the relationships between OCs in different compartments, this is not a concern. The same applies to other confounders, such as parity, smoking, age and diet (see below). The observed DDE : DDT ratios of 8.9 for MM and CP and 9.8 for MP may be designated as mid-range compared to values reported worldwide: 35.0 (Sweden), 28.6 (Iceland), 26.0 (Norway) and 16.8 (Canada).[1,23] Interestingly, other areas in Russia appear to have even lower ratios, 8.2 (Nikel)[1] and 4.7 (Arkhangelsk)[23] than those observed in the present study, while Uelen (also in Chukotka) had a reported ratio of 15.[24] A study completed in Kenya, where there is known current use of DDTs, reported a DDE : DDT ratio of about 1.5 in maternal plasma.[25] Considering that the half-life of *p,p′*-DDT (around 4 years)[6,26] appears to be shorter than for *p,p′*-DDE (6–13 years),[6,26,27] it seems appropriate to suggest[4] that there had been some recent exposure to DDT in the communities studied. For many arctic communities, exposure to DDT and other OCs is primarily determined by long-range transport to northern latitudes and long-term environmental persistence.[28,29]

The detection frequencies summarized in Tables 3–5 clearly show that MM is the most sensitive compartment when considering the proportion of OCs present, while CP was the least sensitive. The latter has been noted previously.[1,9] Further, the reason why the 80% DF was not applied to the sum of the different families of OCs was that they reflect the major members of a group. Perhaps the relatively young age of the mothers might have contributed to the findings that DF $<$ 80% in a relatively large number of OCs, as age is predictive of accumulated exposure.[21,30]

Lipid levels

The lipid-levels in MP and MM were on average 0.54% and 3.07%. These levels are consistent with results in other publications.[8,24,31] Of special interest is the variation and the range of the data within each compartment. While MP is fairly uniform with a V of 0.22, there is more variation in the lipid content in MM (V = 0.36). The milk was sampled between days 14 and 21 after delivery, so the most active changes in milk composition (which occurs during the first 10 days)[32] were avoided. On the other hand, the fat content in the milk is also highly dependent on the time since the breast was last emptied and to which degree it was emptied before sampling,[33] as well as the lactation period and seasonal variations.[25] For the low concentration phase of the lipids in CP, levels were close to the DL and an overestimation of the lipid-adjusted concentrations was feared. The seven CP samples with the elevated lipids had concentrations comparable to those observed in MP. It was suspected that contamination with maternal blood and/or lipids from the umbilical vein/artery walls had occurred. This possibility contributed to the decision not to use the lipid-adjusted CP values, even though they are reported in Table 5B . Compared to MP, a lack of uniform collection time and handling protocols is potentially larger for MM and CP.[34]

Recent studies[35,36,37] use standard enzymatic methods to determine the total lipid content of plasma. The use of the Needham *et al.*[38] formula to calculate total lipids in such assays appear to yield values that are 15–20% higher than those obtained by the gravimetric method.[39,40] This illustrates that the absolute values of lipid-adjusted PTS concentrations depend on the analytical method employed in the assessment of total lipids. Further, if the variations in the fat content are considered to be an important physiological measurement to be taken into account in any statistical analysis, the time-restrictions on milk collection and the potential of contamination during cord blood retrieval support the notion that MP is the most uniform medium available.

Intercompartmental *r*-values

The ratios of V values for unadjusted OCs concentrations in MP and MM were for the most part close to 1 : 1 (V values were numerically somewhat higher in MM). The comparable ratio for the same OCs for MP or MM and CP was typically 1 : 2. Fat adjustment increased V slightly for MP and MM.

The general trend of only a slight decrease in r-values for the lipid-adjusted calculations might imply that the regression analyses are very robust and that it is of no great importance to lipid-adjust when considering the strength and the direction of a linear relationship between the compartments. On the other hand, it could mean that the somewhat lower r-values for the lipid-adjusted comparisons actually constitute a better inherent fit. Population variations should also be considered. Sandanger *et al.*,[40] who compared non-fasting with fasting concentrations of OCs as well as the effect of lipid intake on the latter, recommend that both wet-weight and lipid-weight concentrations should continue to be reported and that the fasting condition should prevail.

As already indicated, r-values in the present set of results had to exceed a value of 0.4 to maintain a statistical power of 0.8 at the 95% level of significance. A perusal of the magnitude of the correlation coefficients reported in Table 6 indicates that this goal was achieved for all intercompartmental correlations examined, except for Cd between MB and CB (r = 0.05). This, and the relatively low level of Cd in CB, supports animal experiments, case control studies[41] and human cohort studies[42] that a placental barrier exists for this metal.

Most of the PCB congeners were highly correlated across the compartments, although less so for congener 118 and 156. Both exhibited a relatively high number of non-detects

compared to the rest of the congeners (see Table S1‡). When we removed all pairs with one or both values below the DL for the OCs eligible for this exercise, the *r*-values changed little for the intercompartmental comparisons (MM and MP) except for PCB-156. In this case the *r*-value went up from 0.55 to 0.79, as illustrated in Fig. S6 and S7‡. This was attributed to the removal of 5 pairs with low correlation. However, caution appears warranted in interpreting this improvement in the correlation as Duval and Karlsson[43] warn that bias may be introduced when omitting a large fraction of data below the DL in a two-compartment model. In all, our findings suggest that the correlation analyses between compartments were generally robust and not very sensitive to whether numbers were replaced, removed or lipid-adjusted.

Pb was detected in all maternal and cord whole-blood samples. Interestingly, Cd was present in 72.9% of the cord blood and in 100% of the maternal whole-blood samples. As already indicated, attenuation of Cd by the placenta is well established,[41,42] as well as the robust relationships observed between maternal and cord whole blood concentrations of Pb or Hg.[9,44,45]

Intracompartmental *r*-values

As others have observed,[9] the OCs were extensively inter-correlated for all three compartments. For the PCB congeners, somewhat lower *r*-values were observed for PCB-118 in MP (Table S3‡) and PCB-156 in MM (Table S2‡). Ayotte *et al.*[35] saw a similar trend for PCBs-105 and 118 in the plasma for 40 members of a coastal fish-eating population, and interpreted this anomaly to the dioxin-like activities for these two congeners. In fact, PCB-156 also exhibits such activity.[46] However, an inspection of the DF values in Table S1‡ indicates that all three congeners in our study had a considerable number of non-detects. As described above for PCB-156 for the intercompartment MP–MM comparison, it seems reasonable to attribute the lower inter-congener *r*-values to mismatched data pairs.

Among the OC pesticides, the DDTs, *p*,*p*′-DDE, *p*,*p*′-DDD and *p*,*p*′-DDT stood out by being poorly correlated with all other OCs tested, except α- and β-HCH (see Table S2‡). This is consistent with the suspicion that there are local sources of DDT, and this may also be the case for β-HCH.[1]

Of special interest is the association between Pb and Cd in MP. Though the primary source of Cd is cigarette smoke, smoking may also constitute a minor exposure risk factor for Pb.[47,48] The relatively moderate values for the Pb associations with ∑PCBs ($r = 0.31$), aroclor ($r = 0.35$), ∑DDT ($r = 0.21$), and HCB ($r = 0.35$) are somewhat surprising (Table S3‡). It perhaps signifies that some of the traditional foods harvested by hunting with leaded ammunition, such as terrestrial animals (*e.g.* reindeer) and birds (*e.g.* fish-eating birds and bottom-feeding waterfowl), constitute not only dietary sources for Pb,[49,50] but also for intake of OCs. For example, there is some evidence that dabbling ducks and non-marine mammals constitute potential exposure pathways for OCs (Nieboer, unpublished results).

Conclusions

1. Maternal plasma (MP) is the most fundamental biomonitoring medium for organochlorines (OCs) for the following reasons: mother's milk (MM) OC levels correlate with MP values; the transfer of MP lipids to the fetus appears to be regulated by the maternal–fetal concentration gradient[51] and the same metabolic driving force likely applies to OCs, making MP OC concentration the primary exposure index for the fetus; MP lipids and OC levels are likely also the general driving force for MM lipids and OC concentrations,[52] and thus indirectly also the exposure to OCs experienced by the breast-fed neonate. However, MP concentrations as an exposure index is of an intermediate sensitivity (based on concentrations, number of OCs and detection frequency), while MM is the most sensitive. MM collection is, nonetheless, somewhat intrusive and inaccessible (in terms of timing after birth) and has some dependence on the penultimate feeding. Cord plasma (CP) is the least sensitive exposure index in terms of OC concentrations, detection frequency, and the collection of specimens itself is also challenging.

2. There was no apparent improvement in *r*-values after lipid adjustment for comparisons between MM and MP in the cohort studied.

3. Even though we selected an 80% detection frequency (DF) as the limit, the literature and theoretical arguments discussed suggest that any DF below 90% may introduce bias. The ultimate use of the data may need to be considered in the decision of how large a percentage of non-detects should be allowed. In the case of the present study, whether it was 80 or 90% proved to be of no consequence for the observed *r*-values.

4. The substitution of concentrations below the detection limit (DL) requires inspection of the data and fulfilment of certain criteria, such as skewness and geometric standard deviation (GSD).

5. The variation observed in the lipid content of CP needs further attention. Cord blood (CB) sampling protocols need to ensure that contamination of the sample with maternal and cord tissue lipids is avoided.

List of acronyms

AM:	Arithmetic mean
AMAP:	Arctic Monitoring and Assessment Programme
CAO:	Chukotski Autonomous Okrug (Region)
CB:	Cord blood
CEC:	Center for Environmental Chemistry
CP:	Cord plasma
DF:	Detection frequency
DL:	Detection limit
GM:	Geometric mean
GSD:	Geometric standard deviation
MB:	Maternal blood
MM:	Mothers milk
MP:	Maternal plasma
QA:	Quality assurance
QC:	Quality control

(*continued*)

r:	Pearson's regression coefficient
S:	Appears in the ESI‡
SD:	Standard deviation
SPA:	Scientific Production Association
V:	Coefficient of variation

List of chemical abbreviations

p,*p*′-DDD:	*para*,*para*-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
o,*p*′-DDD:	*ortho*,*para*-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
p,*p*′-DDE:	*para*,*para*-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethene
o,*p*′-DDE:	*ortho*,*para*-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethene
p,*p*′-DDT:	*para*,*para*-1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
o,*p*′-DDT:	*ortho*,*para*-1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
DCM:	Dichloromethane
HCB:	Hexachlorobenzene
α-HCH:	alpha-Hexachlorocyclohexane
PTS:	Persistent toxic substance
β-HCH:	beta-Hexachlorocyclohexane
γ-HCH:	gamma-Hexachlorocyclohexane
ToxP:	Toxaphene Parlar
OC:	Organochlorine
PCB:	Polychlorinated biphenyl
∑PCB:	Sum of specified number of chlorobiphenyl congeners
Cd:	Cadmium
Hg:	Mercury
Pb:	Lead

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