Lipids and PCDD/Fs in Human Blood Fractions

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Introduction

Human blood is one of the most obtainable objects for the assessment of individual, cohort and population exposure. Extraction of lipids is the first stage of PCDD/Fs determination in blood. The term "lipids" is a working name for the fraction received as a result of extraction of biological tissue by a mixture of polar and non-polar solvents. Using extraction mixtures of different polarity it is possible to extract different fractions of lipids even from plasma: triglycerides, cholesterol, their ethers, phospholipids, etc.

Henderson L. (1) have been established, that 80% of H^3 -TCDD were registered in lipoproteins of plasma, 15% - in pre-albumin of plasma and 5% - in cell elements (in vitro). Patterson's (2) experiments in vivo on PCDD/Fs distribution in blood fractions stated that 9% of PCDD/Fs were in blood cells. Schecter A. and Ryan J. determined the distribution of PCDD/Fs between plasma and cells for 9 individuals as 85/15; and it is within the range of 54 – 90% in plasma for different isomers (3). The extraction system was not named but the data given in (3) made it possible for us to calculate percentage of lipids extracted from plasma and cells. It was 0.26-0.54% for plasma and 0.05-0.14% for cells. Ryan J. [4] considers that formed elements of blood under conditions of extraction serve only as a diluting component for PCDD/Fs determination. The same author in (5) pays attention to the ratio ethanol-hexane (actually, to the polarity of the solvent) in order to provide the total extraction of lipids, but not PCDD/Fs from whole blood.

At the same time it is known that membranes of blood cells (erythrocytes) contain over 40% of lipids but these are the lipids of another class (phospholipids, glucolipids, etc.). A more polar extractant is required for their extraction. Methods of lipid extraction from cell membranes (6, 7) are based on the application of 20 volumes of the mixture chloroform/methanol (2/1) to the volume of the sample, and this allows to extract more than 99% of lipids from cell membranes.

Objects and methods

Ten series of experiments on lipid extraction and PCDD/Fs determination in fractions of serum and formed cells (series I-III), lipid extraction from whole blood of highly exposed donors with the use of different mixtures of solvents (series IV-VII), and also of plasma, thrombocytes and erythrocytes received as a result of separation of fresh

blood samples (series VIII-X) on the centrifuge Sorwal in the Centre of Blood Transfusion (Ufa) were carried out.

The pattern of extraction and the results of lipid and PCDD/Fs determination are given in Tables 1-6. Determination of PCDD/Fs in lipid extracts was carried out according to the method of EPA 1613. All criteria of quality control specified by the methods were observed.

Series I-III. The fractions of blood for 3 pool samples from donors with normal level of lipids are used.

Table 1. Lipids and PCDD/Fs in plasma and "cells"

Fraction	Plasma + $(NH_4)_2SO_4$, aq.	Cells + $(NH_4)_2SO_4$ aq.
Extraction mixture	EtOH:Et_2O:C_7H_{14}=1:1:2	EtOH:Et_2O:C_7H_{14}=2:1:2
Volume, %	33	67
Lipids, %	53	47
TEQ,%	51	49

Series IV-VII. To reduce the error in PCDD/Fs determination a pool sample of whole blood taken from highly exposed donors was used for analysis. The sample was divided into aliquots of 40 ml. Parallel determinations were performed for all series.

Table 2. Patters of extraction from whole blood

40 ml whole blood + C^{13} PCDD/Fs+ 40 ml $(NH_4)_2SO_4$, aq.				
Series	**IV**	**V**	**VI**	**VII**
1-st extraction	40 ml $(CH_3)_2O$, 20 ml C_7H_{14}	40 ml i-C_3H_7OH, 40 ml C_7H_{14}, 20 ml Et_2O	40 ml EtOH, 40 ml C_7H_{14}, 20 ml Et_2O	160 ml CH_3OH, 80 ml $CHCL_3$
2-nd, 3-d extraction	20 ml $(CH_3)_2O$, 20 ml C_7H_{14}	20 ml C_7H_{14}, 20 ml Et_2O	20 ml C_7H_{14}, 20 ml Et_2O	160 ml CH_3OH, 80 ml $CHCL_3$

Extraction by the pattern IV gives % lip. = 0.345±0.049 (n=13), pattern V gives practically the same result. Pattern IV can be applied for plasma, but not for cells or whole blood analysis because it does not provide full extraction of lipids from cell membranes.

The use of classical pattern of lipid extraction from cell membranes (pattern VII) gives the results exceeding by 1.5 times: % lip. = 0.562 + 0.181 (n=8). This certainly has some effect on re-calculation of PCDD/Fs concentration in relation to the weight of lipids.

Series (VIII-XI)

Table 3. The formed blood elements extraction pattern

Series	VIII	IX	X	XI
Fraction	Plasma (n=3)	Eritrocytes(n=3)	Trombosytes(n=3)	Whole blood
% (volume)	20-30	60-65	10-15	100
Sample, ml	40	40	40	40
1-st extraction	10 ml EtOH, 10 ml Et_2O, 15 ml C_7H_{14}	40 ml EtOH , 10 ml Et_2O, 40 ml C_7H_{14}	40 ml EtOH, 10 ml Et_2O, 40 ml C_7H_{14}	40 ml EtOH, 10 ml Et_2O, 40 ml C_7H_{14}
2-nd and 3-d extraction	10 ml Et_2O, 10 ml C_7H_{14}	20 ml Et_2O, 20 ml C_7H_{14}	20 ml Et_2O, 20 ml C_7H_{14}	20 ml Et_2O, 20 ml C_7H_{14}
Lipids,%(w)	0,335	0, 310	0,532	0,345
Lipids, %	26,08	58,70	15,22	100
TCDD,%	59,47	34,44	6,09	100
TEQ,%	54,26	33,67	12,07	100

Table 4. PCDD/Fs in cell fractions, pg/g lipids

PCDD/Fs	Plasma	Erythrocytes	Thrombosydes
2378-TCDD	26.01±5.76	16.79±0.89	2.78±0.42
12378-PnCDD	24.29±6.93	5.53±4.43	2.74±0.93
123478-HxCDD	4.99±3.09	6.4±0	2.3±0.53
123678-HxCDD	17.56±3.57	10.71±0.69	4.64±2.75
123789-HxCDD	4.45±2.87	4.32±1.82	2.11±0.46
1234678-HpCDD	13.87±2.72	13.45±3.41	6.47±1.05
OCDD	80.26±9.33	95.99±5.9	44.17±0.21
2378-TCDF	10.26±0.03	11.65±0.52	4.96±0.68
12378-PnCDF	15.89±3.85	13.52±17.42	11.15±2.38
23478-PnCDF	34.42±8.68	20.77±0.03	12.2±0.77
123478-HxCDF	44.20±10.03	50.41±2.84	22.48±0.99
123678-HxCDF	14.81±3.00	12.85±1.63	7.49±2.07
123789-HxCDF	15.88±2.07	22.01±1.03	10.02±0.36
234678-HpCDF	10.24±0.81	1.81±0.05	5.66±0.45
1234678-HpCDF	18.71±5.24	22.63±2.74	10.22±1.47
1234789-HpCDF	11.14±1.61	17.88±0.16	5.36±0.37
OCDF	17.39±2.81	39.91±1.4	9±3.1
Lipids, g (40 ml)	0.135±0.015	0.124±0.02	0.213±0.09
PCDD/Fs, TEQ, pg/g	68.9±15.13	47.8±2.26	17,06±0,93

Results and Discussion

Using extractants of higher polarity we succeeded in total extraction of lipids and PCDD/Fs from membranes of non-damaged formed elements of human blood. Concentration of lipids and PCDD/Fs in cells approximately corresponds to the concentration in plasma by the absolute content, or plasma/cells = 1.5/1, taking into account the volume distribution in whole blood. PCDD/Fs are mainly found in membranes of erythrocytes, probably due to extremely developed surface of these cells. Thrombocytes contain inconsiderable amount of PCDD/Fs, their contribution to blood lipids is insignificant due to their small share in blood.

Obviously permanent exchange of lipids (and PCDD/Fs) in membranes of erythrocytes and plasma is going on because the lifetime of erythrocytes in human blood is only 100-120 days.

References

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Table 5. PCDD/Fs in plasma and cells

PCDD/Fs	Plasma			Cells			% in plasma
	Pg	Ppt, lipid	Ppt,whole	pg	Ppt, lipid	Ppt,whole	
2378-TCDD	3.28±1.41	20.23±4.73	0 12±0.05	2.14±0.68	16.61±2.36	0.05±0.01	53.53±5.53
12378-PnCDD	3.58±2.87	18.79±7.8	0.11±0.05	3.46±2.87	23.09±10.84	0.07±0.05	45±12.69
123478-HxCDD	1.54±1.34	8.85±5	0.06±0.06	0.8±0 55	7.61±0.41	0.02±0.01	49.13±17.11
123678-HxCDD	1.99±1.43	9.35±5.85	0.07±0.06	2±1.15	8.26±6.31	0.04±0.02	41.97±11.9
123789-HxCDD	1.19±0.85	6.75±2.73	0.04±0.04	1.03±0.71	7.38±2.4	0.02±0.01	46.13±9.57
1234678-HpCDD	3.46±1.32	19.33±12.97	0.11±0.09	2.82±2.64	18.26±10.75	0.06±0.05	55.46±15.16
OCDD	21.08±11.43	122.42±25.20	0.59±0.62	13.57±9.73	94±27.84	0.29±0.18	55.74±2.64
2378-TCDF	1.36±0.67	11.82±11.39	0.05±0.03	1.26±0.79	10.51±8.79	0.03±0.02	49.0±22.77
12378-PnCDF	0.81±0.39	7.11±7.35	0.03±0.03	0.83±0.32	6.5±3.18	0.02±0.01	43.64±16.08
23478-PnCDF	3.03±1.13	19.20±5.23	0.11±0.02	1.64±0.24	13.13±3.09	0.04±0.01	50.29±17.1
123478-HxCDF	1.72±0.96	9.69±4.13	0.06±0.04	1.81±0.77	13.69±1.99	0.04±0.01	41.49±7.42
123678-HxCDF	1.28±0.58	6.95±1.77	0.05±0.02	1.36±0.95	9.46±2.7	0.03±0.02	59.47±16.14
123789-HxCDF	0,75±0,19	6.75±3.56	0.03±0.02	1.64±0.35	4.69±0.7	0.02±0.01	43.20±19.27
234678-HpCDF	1.25±0.15	8.82±5.46	0.05±0.02	1.22±0.63	9.09±2.2	0.03±0.01	46.65±5.73
1234678-HpCDF	2.34±1.21	14.15±3.96	0.09±0.05	3.18±1.52	24.1±6.52	0.07±0.03	36.36±6.26
1234789-HpCDF	1,35±0,78	13.09±15.55	0.06±0.06	1.35±0.69	10.65±6.05	0.03±0.02	33.71±6.18
OCDF	3.13±2.18	16.88±4.7	0.31±0.38	2.11±1.29	15±4.94	0.05±0.02	52.5±4.61

Table 6. The solvent polarity influence on lipids and PCDD/Fs extraction from whole blood samples.

PCDD/Fs	Series IV		Series V		Series VI		Series VII	
	Ppt, lipid	Ppt, whole	Ppt, lipid	Ppt, whole	Ppt, lipid	Ppt, whole	Ppt, lipid	Ppt, whole
2378-TCDD	214.04±22.29	0.29±0	116.7±0.71	0.36±0.01	93.22±14.28	0.4±0.02	66.48±0.6	0.36±0.01
12378-PnCDD	366.31±59.25	0.51±0.01	213.66±10.72	0.66±0.02	153.95±14.21	0.65±0.01	123.71±1.12	0.67±0.03
123478-HxCDD	54.03±6.18	0.07±0	33.6±5.2	0.1±0.01	27.07±1.51	0.12±0.01	17.57±0.46	0.1±0.01
123678-HxCDD	285.05±27.37	0.39±0.01	175.44±3.06	0.54±0	134.59±22.89	0.57±0.04	102.43±0.13	0.56±0.02
123789-HxCDD	124.16±13.92	0.17±0.01	74.96±3.51	0.23±0.01	50.84±6.6	0.21±0	34.89±2.65	0.24±0.01
1234678-HpCDD	345.92±42.26	0.47±0.01	207.87±8.75	0.64±0.01	159.86±29.5	0.68±0.05	125.18±5.74	0.68±0
OCDD	2258.87±217.12	3.03±0.03	1497.09±50.98	4.65±0.01	1129.9±1121	4.78±0.01	896.74±19.4	4.85±0.1
2378-TCDF	24.94±4.05	0.03±0	16.06±0.15	0.05±0	11.34±0.09	0.05±0.01	9.59±1	0.05±0
12378-PnCDF	70.97±18.43	0.1±0.02	42.95±1.49	0.14±0.01	25.48±0.88	0.11±0.01	21.27±4.27	0.12±0.02
23478-PnCDF	125.25±20.58	1.17±0.01	73.21±0.01	0.23±0.01	55.7±4.42	0.24±0.01	43.36±2.73	0.24±0.01
123478-HxCDF	203.05±39.24	0.27±0.03	128.31±5.45	0.4±0.02	87.82±14.38	0.37±0.01	81.86±4.79	0.45±0.01
123678-HxCDF	86.51±12.86	0.12±0.01	51.63±0.38	0.16±0	39.55±6.56	0.17±0.01	40.97±2.14	0.24±0.02
123789-HxCDF	74.27±16.03	0.1±0.01	43.7±3.25	0.14±0.01	30.43±1.08	0.13±0.01	21.99±2.43	0.12±0.01
234678-HpCDF	49.29±5.82	0.07±0	31.28±1.81	0.1±0.01	20.59±2.21	0.09±0	20.72±1.2	0.11±0
1234678-HpCDF	219.42±17.23	0.3±0.01	140.45±7.43	0.43±0.01	101.2±6.49	0.43±0.01	145.35±3.68	0.79±0.02
1234789-HpCDF	45.74±5.18	0.06±0	30.87±2.28	0.1±0.01	21.95±6.15	0.09±0.01	18.95±1.75	0.11±0.01
OCDF	104.04±45.21	0.14±0.05	63.53±6	0.2±0.01	33.69±8.08	0.14±0.01	88.56±0.69	0.48±0.01