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RĪGAS STRADIŅA UNIVERSITĀTE

Pāvels Sudmalis

POSSIBILITIES OF PERSISTENT ORGANIC POLLUTANTS DETERMINATION AND DETECTION IN ENVIRONMENT AND BIOLOGICAL SAMPLES

Summary of Doctoral Thesis
Speciality – Pharmacy
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CONTENTS

Introduction

Topicality of the problem

Persistent organic pollutants (POPs) are not nature products; they are human-created organic substances that contain halogens (chlorine, bromine); their chemical stableness prevents their degradation in nature for decades. POPs are among the most dangerous pollutants created by human, since they cause irreversible effect such as inherited vices, for example, or cancer, allergies, and exhausted immune system. As a result of wind and water movement POPs are moved to a very large distance from the initial sites of production or use. According to the source and application POPs can be divided into three smaller groups:

- pesticides - dichloro diphenyl trichloroethane (DDT) and its metabolites, dieldrin, endrin, hexachlorocyclohexane, chlordane, chlordecone, toxaphene, etc.;
- substances used in industrial raw materials: Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), hexachlorobenzene, etc.;
- high-temperature processes products: polychlorinated dibenzo-p-dioxins and dibenzofurans (PHDD/F) (*Latvijas Republikas Vides ministrija*, 2005; *Veselības inspekcija*, 2012).

It is known that pesticides are one of the main persistent organic pollutants. The chemicals used in agriculture, as well as chemical weapons are the only substances in the environment with a single mission - to kill living creatures. Thus, they pose major risks to both human health and the environment. Information about the total imported DDT in Latvia is insufficient. According to the National Environment Service and the State Plant Protection Service data in1961-1967 year period 367 tons of DDT were used in Latvia. The main use of DDT was: as an

insecticide – for sugar beet, potato and cereal forage crops protect, fruit and vegetable pest control, as a biocide - to fight against livestock parasites. Since 1968, use of DDT is prohibited in Latvia, but it can still be detected in the environment. Now in Kņavas and Gardenes hazardous waste disposals are located more than 1860 tons of hazardous chemical waste, of which about 200 tons of DDT and more than 200 tons of pesticide mixtures, containing lindane and DDT *(Latvijas Republikas Vides ministrija*, 2005). However, this does not mean that all of DDT and its derivatives containing mixtures and products were collected and stored under appropriate conditions.

Polychlorinated biphenyls (PCBs) are one of the most dangerous and persistent organic pollutants present in Latvia. PCBs are also among the most widespread environmental contaminants because they are found almost everywhere (indoor and outdoor air, soil, groundwater and surface water, food). In nature pollution occurs discarding the of PCB-containing equipment. PCB are evident, in the disruption of homeostasis balance (*Klavins & Roska*, 1998; *Barbalace*, 2003, *Valsts vides dienests*, 2005).

Polybrominated diphenyl ethers PBDE are brominated organic compounds used as flame retardants. They are used for a wide range of products: construction materials, electronic goods, furniture, in motor vehicles, airplanes, plastics, polyurethane foams and textiles (*Kemmlein*, 2003; *Stapleton*, 2006). By its structure they are similar to polychlorinated biphenyls which production and use is prohibited because of their toxicity. PBDEs have been put forward for inclusion in the Stockholm Convention banned substance lists. There is evidence that PBDEs persist in the environment and bioaccumulate in the blood, breast milk and adipose tissue, they can reduce fertility. The main entry routes of PBDE in the body is through inhalation, ingestion of contaminated food and ingestion of dust (*Thomasen*, 2001; *Fredericsen*, 2010).

As study target group selected Latvian enterprises were employed electricians, they performing their work duties they may have contact PCB and PBDE-

containing products (transformer oil, powerful capacitors, plastic and other materials that may contain flame retardants). The second target group - mothers living in Olaine. Olaine is one of the most polluted Latvian cities because several chemical companies are located in this area.

Complex research of the level of POPs in different biological environments and in ambient air should be conducted in Latvia to identify the exposition of population to the given substances. Little attention has been paid to the given topic in Latvia until present, and therefore little information is available about the harm eventually caused by the persistent organic pollutants to human health. Data obtained in this research would enable understanding of the levels of POPs in electricians and welders employed by Latvian enterprises, as well as the level of POPs present in breast milk of moters and the degree of risk eventually posed to newborn children. The presence of POPs in ambient air and on working surfaces would also be identified.

Electricians employed by Latvian enterprises have been selected as the target group for research since their direct job duties may involve contact to products that contain PCBs and PBDEs (transformer oil, powerful capacitors, plastics and other material that may contain flame extinguishers). Young mothers residing in Olaine have been selected as another target group because of a number of chemical plants situated in Olaine; this location is therefore treated as one of the most polluted locations in Latvia.

Purpose of the Paper

To identify relations between the concentrations of POPs in biological environments of human bodies and their subjective health condition; to compare the collected data with the levels in other countries, as well as to assess the potential risk to human health, and the eventual pollution of working environment and food supplements with POPs.

Objectives of work

To summarize and analyze information about the levels of POPs in different environments, their effect on human body, and the methods for their detection;

To identify and adapt appropriate, scientifically substantiated methods us chromatography for detection quantification of POPs in biological matrixes and in the environment;

To identify the employees and young mothers eventually exposed to the effect caused by POPs; to conduct a survey among them in order to identify their subjective health condition;

To collect biological material, subject to consent of respondents to participate in research, and to determine the concentrations of POPs;

To identify the relation between the concentrations of polluting substances in biological environments and the results of the survey;

To assess working environment in order to detect the presence of POPs, and to compare the obtained data with the results of analysis of biological samples;

To consider food supplements (cod-liver oil) as the potential source of POPs.

Hypothesis of the work

The levels of persistent organic pollutants (POPs) present in population are not sufficiently identified in Latvia, and no appropriate methods are adapted to analyze different types of biological samples.

Scientific novelty of the work

Complex research of the levels of POPs present in human milk, blood of employees, ambient air at working place and food supplements is conducted for the first time in Latvia.

Scientifically substantiated methodology is developed for qualitative and quantitative detection of different POPs in biological and environmental samples.

Correlation has been established between the levels of POPs in biological samples and physiological parameters of human body.

1. MATERIALS AND METHODS

1.1. Analyzed Samples

The Paper has been developed at the Laboratory of Hygiene and Occupational Diseases and Faculty of Pharmacy of Riga Stradin's University (RSU). The work has been supported from the program "Support to doctoral students in completing the program and gaining the scientific degree of Doctor from Riga Stradin's University", within the framework of scientific project "Research of Exogenous and Endogenous Factors Endangering the Health of Latvian Residents" subproject RSU ZP08/03-2 "Impact of Metal and Stable Organic Pollutants on the Activity of Antioxidants for Employees in Certain Latvian Industrial Enterprises" for the period from 2006 to 2008, and within the scope of the UNDP/GEF project "Drafting of the National Implementation Plan for Persistent Organic Pollutants within the framework of the Stockholm Convention", contract No VP 406/167.

The pursued work included analysis of four different groups of samples to detect presence of persistent organic pollutants. 30 samples of human milk were analyzed (15 representing the target group and 15 the control group samples, respectively), 116 samples of blood serum (25 of electricians' and 91 of welders' blood, respectively); 15 Latvian enterprises were tested to detect the presence of POPs in the working environment (collected air samples and surface wash-offs); as well as analysis of 5 different forms (capsules, castable) and food supplements from manufacturers (cod-liver oil) intended for different age groups.

1.2. Identifiable compounds

Presence of 28 persistent organic pollutants was detected in all samples.

Analysis was performed on 18 fixed representatives from three different groups of PCBs to ensure objective assessment of pollution of the samples with polychlorinated biphenyls: Marker – PCB (CB-28; CB-52; CB-101; CB-138; CB-

153; CB-180), Mono-ortho – PCB (CB-105; CB-114; CB-118; CB-123; CB-156; CB-157; CB-167; CB-189) and Non-ortho – PCB (CB-77; CB-81; CB126; CB-169).

Four compounds of flame extinguisher group were identified in the samples: BDE-47, BDE-99, BDE-100 and BDE-153, as well as six pesticides: o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT.

1.3. Sample preparation

For quantitative determination of persistent organic pollutants in biological matrix at we compared two sample preparation methods with different variations of the solvent (methanol, isopropanol). Methods were compared using breast milk samples from a single donor. The comparison was done by CB - 174 recovery because the given substance under normal conditions does not accumulate in the body.

1.3.1. Biological sample preparation method using a simple extraction

This sample preparation method was chosen because it allows operating with a larger sample volume and thus the percentage of operator error will be smaller.

A day before analysis milk was thawed. In analysis container 50 ml of sample and add 50 microliter of internal standard CB -174 with a concentration of 500 μg/ml. At next day, milk with internal standard quantitative transferred to 250 ml separating funnel. Potassium oxalate/water solution prepared by dissolving 0.5 g of potassium oxalate monohydrate in 10 ml of hot water was added, and immediately 50 ml of ethanol, 20 ml of diethyl ether and 30 ml of hexane also added, after the last component added shall two minute performed extraction. After the organic phase separation the aqueous phase was extracted twice more with 30 ml of hexane. All organic fractions combined were transferred to separating funnel, 5 ml of concentrated sulfuric acid added and mixed two minutes, which were repeated

twice. Extract is then quantitatively transferred to rotor evaporator flask and evaporated to about 2 ml.

Next sample preparation step is the treatment of solid-phase extraction method, which is common to all sample preparation methods. In this step of sample purification it was passed through a column filled with 1 gram of purified and activated fluorosil, and 1 g of 44% sulfuric acid silica gel mixture (prepared by mixing 40 grams of activated silica gel with 19 ml of 96% sulfuric acid) and 1 g of anhydrous sodium sulfate placed on the top of the column. Column was activated with 8 ml of hexane and immediately sample was introduced, followed by elution using 10 ml of hexane. To the obtained extract 10 µl of iso-octane was added and it was evaporated almost to dryness. The residue was dissolved in 100 µl of iso-octane and transferred to 2 ml gas chromatography vial with 250 µl inserts.

1.3.2. Biological sample preparation method using solid phase extraction

A day before purification the samples were thawed and internal standard (CB 174) was added. The samples were purified by double solid-phase extraction. Before extraction of the sample 10 ml methanol was added and 10 minutes homogenization in ultrasonic bath was done. Extraction was carried out with Supelco company produced solid phase extraction tubes "Supelclean LC-18 SPE Tubes 6ml (1gm). The column was inserted in industrially produced solid phase extractor, with the possibility of simultaneous extraction of 12 columns, using vacuum. The columns were conditioned with 10 ml of methanol and 20 ml of water. Sample immediately quantitatively transferred into column. Followed by ad 5 ml of methanol and kept for one hour in vacuum to dry and then POPs eluted with 10 ml of hexane-dichloroethane mixture (9:1). To break down fats present in the extract, 0.5 ml of concentrated sulfuric acid was added and shaken for 2 minutes.

Given the fact that methanol is highly toxic solvent, attempts were made with less toxic isopropanol to determine whether it can be used instead of methanol.

1.3.3. Environmental sample preparation for gas chromatographic analysis

Working environment air samples were taken in the workers breathing zone over the day with individual air-sampling devices "Gilian LFS - 113DC", on carbon tubes "ORBO TM - 32" and with Gilian 5, on Milipore filters. From the surface (0.16 m2), dust and other impurities were taken with medical cotton swabs.

The simplest and fastest samples preparation was from the activated charcoal. IT was done with 0.5 ml of carbon disulphide in an hour.

From Milipore filters and cotton swabs POPs were extracted by continuous distillation plant with 50 ml of iso-octane. Extract was evaporated in rotor vaporizer to about 2 ml. Further extract evaporation to about 0.5 ml was done by air in solid phase extraction device.

1.4. Data collection and statistical analyses

POPs levels in the samples are calculated using the calibration graphs and the internal standard recovery level.

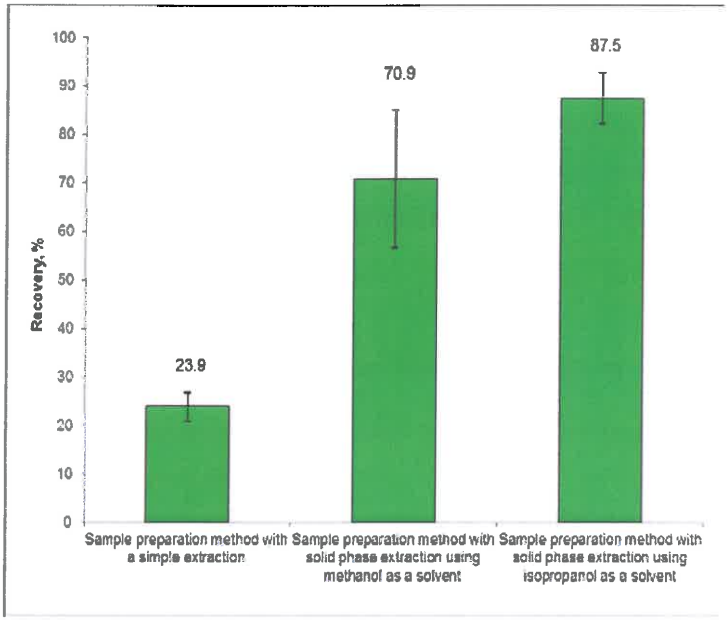
Statistical Results Processing was performed by using SPSS 16.0, Microsoft Excel and MedCalc 12.2.0.0 computer programs were used in separate cases. Generally recognized statistical methods (*Teibe & Berķis*, 2001; *Paura & Arhipova*, 2002; *Teibe*, 2007) were used in data statistical analysis. Consistency of acquired research results to normal (Gauss) division was examined by using Kolmogorov-Smirnov test (K-S test), asymmetry and excess of results were also assessed. Since the measurements of POPs in biological samples did not comply with normal distribution, nonparametric data procession methods were used for statistical processing of results. Corresponding statistical analysis was performed

for each variable quantity – indices of central trends (arithmetic mean, median) and dispersion (standard deviation, 95% confidence interval) were found as well as quartiles calculated. For result comparison Odds Ratio (OR), Chi-square tests, confidence interval (95%) methods of analysis were applied, as well as Mann-Whitney U test, linear regression, Spearman's rank correlation coefficient calculated.

2. RESULTS

2.1. Sample preparation

Two methods for preparing samples with different variations of solvents (methyl alcohol and isoprophanol, respectively) applied to quantify persistent organic pollutants in biological matrixes were compared. The methods were compared on samples of human milk taken from the same donor. Comparison was based on recovery of CB – 174 since the given substance normally does not accumulate in human body (see fig. 2.1).



2.1. fig. Biological sample preparation methods comparison

The results lead to conclusion that preparing of samples by solid phase extraction where isoprophanol is used as the solvent is the best method applicable to preparing of biological samples for gas chromatographic analysis in laboratory environment, since it provides the highest degree of PCB-174 retrievability –

87.5%. This method is simpler and less effort-consuming, and it involves consumption of smaller amount of solvent, compared to preparing of samples by ordinary extraction. The fact that solvent used in this method is less harmful to human health is also important.

2.2. Equipment and reagents used in the work

The conducted research included analysis of samples by means of two gas chromatographers:

„*Varian 3800*" with automated sample input system "*CP – 8200*", ^{63}Ni electronic gripper detector, which is especially selective to organic compounds containing halogens (it as used to detect the quantity of POPs in the samples);

"*Agilent 6890N*" with mass-spectral analyzer "*Waters Micromass*" (for identification of POPs in the samples).

Masspectral analyzes was done in single ion detection mode (SIR), ionization energy was 40 eV see 2.1. table.

2.1. table

Detected ion values

Compaund type	Molecular formula	Detectable m/z value
PCB	$C_{12}H_7Cl_3$	257.54
	$C_{12}H_6Cl_4$	291.98
	$C_{12}H_5Cl_5$	326.43
	$C_{12}H_4Cl_6$	360.88
	$C_{12}H_3Cl_7$	395.32
PBDE	$C_{12}H_6Br_4O$	485.79
	$C_{12}H_5Br_5O$	564.68
	$C_{12}H_4Br_6O$	643.58
DDT	$C_{14}H_9Cl_5$	354.49
DDD	$C_{14}H_{10}Cl_4$	320.04
DDE	$C_{14}H_8Cl_4$	318.02

A certified reference sample of milk *PCB CRM 450 Chlorobiphenyls in natural milk powder, Community Bureau of Reference – BCR* was used to check correctness of the selected method for analysis of samples. It was analyzed in the same way as any other biological samples. Please refer to 2.2. table for results of analysis of the reference milk sample.

2.2. table

Results of analysis of reference milk

PCB by IUPAC	c PCB detected, ng/g	c PCB given, ng/g	Recovery, %
CB-52	15.92	1.16	1372*
CB-118	2.25	3.30	68.1
CB-153	16.24	19.00	85.5
CB-156	1.12	1.62	69.1
CB-180	10.69	11.00	97.1

* PCB-52 concentration results is improbable, the obtained results are attributable to the increased overlapping of the peaks, and this compound test results are not included in milk samples results calculation

According to the table, retrievability is satisfactory in part of the PCBs and low in the other part; steps have therefore been taken according to the results of analysis of the reference samples to improve quality of work. Internal PCB standards (HB-103 and HB-174) have been used to identify the identifiable PCBs and evaluate the degree of their extraction, i.e. PCB, not contained in biological samples since they transform into other PCBs as a result of metabolism processes.

2.3. Analysis of milk samples to detect the presence of POPs

The results of survey conducted among young mothers lead to conclusion that Olaine target group and control group are comparable, and their characteristics show no significant differences. The survey covered 30 young mothers: 15 in Olaine group and 15 in control group, respectively.

Three gas chromatography measurements of the concentration of persistent organic pollutants were concurrently made in respect of each analyzed milk

sample. To facilitate comparison of the relevant POP concentrations in samples obtained from Olaine group and those from control group, the results were summarized in groups according to POP types: marker- PCB, mono-ortho-PCB, non-ortho- PCB and PBDE (see Table 2.3.).

2.3. table

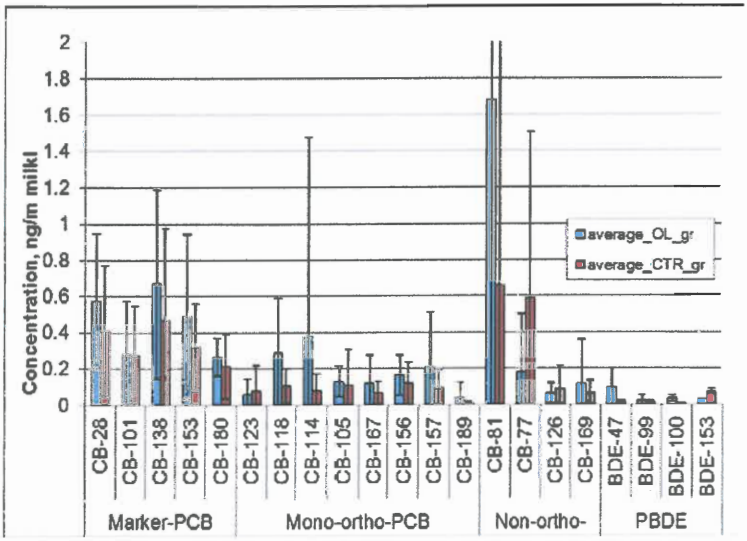
POPs average concentrations (ng/ml) in Olaine (OL) and control (CTR) breast milk samples

Sample	Marker-PCB sum	Mono-ortho-PCB sum	Non-ortho-PCB sum	PBDE sum
OL median	2.048	1.053	0.357	0.005
OL average	2.282	1.385	2.043	0.069
SD	1.260	1.161	4.752	0.075
min. conc.	0.764	0.292	0.051	<0.010[a]
maks. conc.	4.924	5.183	19.246	0.285
95% TI	1.363 – 3.493	0.676 – 1.801	0.214 – 0.990	0.024 – 0.068
IQR ($Q_3 - Q_1$)	1.360 – 3.495	0.675 –1.810	0.214 – 0.990	0.011 – 0.088
OL TEQ pg/ml milk		0.428	7.730	
CTRL median	1.790	0.643	0.301	0.005
CTR average	1.687	0.653	1.400	0.0313
SD	1.140	0.528	1.811	0.033
min. conc.	0.088	0.035	0.014	<0.010[a]
maks. conc.	4.204	2.112	5.503	0.098
95% TI	0.641 – 2.412	0.184 – 0.849	0.180 – 2.763	<0.010[a] – <0.010[a]
IQR ($Q_3 - Q_1$)	0.637 – 2.414	0.184 – 0.849	0.180 – 2.798	<0.010[a] – 0.016
CTR TEQ pg/ml milk		0.174	9.569	
p-value	0.1409	0.0265	0.8846	0.0011

[a]Below detection limits

According to the results of measurements, the quantities of POPs detected by means of gas chromatography represent a highly wide concentration range: from level under the limit for detection of POPs (0.014 – 0.05 pg) to tens of pg.

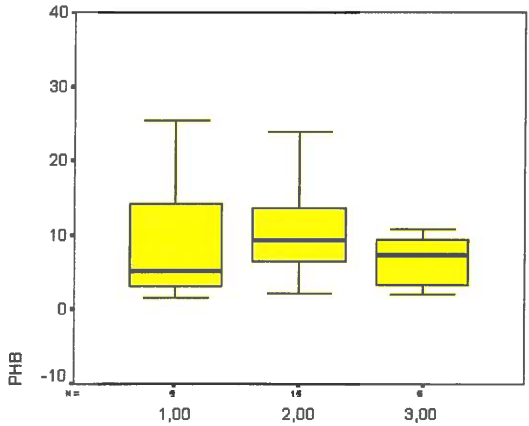
Concentration of each POP in the milk sample was calculated according to the chromatographically measured quantity of POPs, subject to the volume of milk used for extraction and adjustment factor according to the internal standard of HB-174 retrievability. Half of the limit for detection of POPs (rather than zero value) was used as the minimum concentration for calculation of average values to avoid intentional decrease of values. Averages POPs levels are given in fig. 2.2.



2.2. fig. POPs average mass concentrations (γ average, ng/ml) Olaine (blue) and control (brown) group in the milk samples

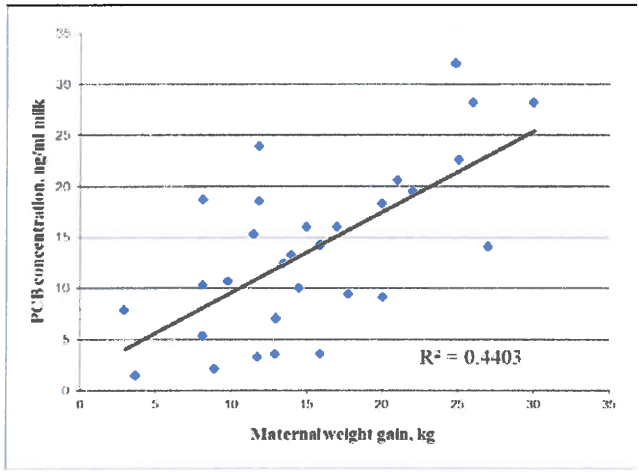
2.3.1. Comparison of the concentration of POPs in milk samples

Comparison of the obtained results and the results of survey identified no correlation between the frequency of fat food consumption and concentration of PCBs in human milk ($p = 0.356$) (fig. 2.3.), where all fat products are grouped in three consumption frequency categories: 1.00 – never, or once a week; 2.00 – twice a week or less; 3.00 – more than twice a week.



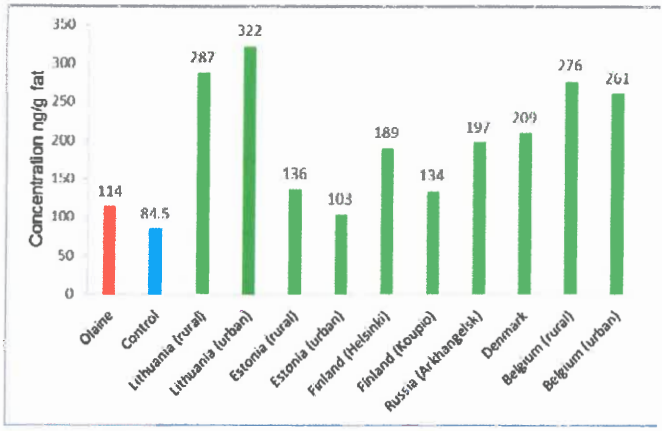
2.3.fig. PCB levels correlation in milk with fatty products consumption

Correlation is, however, statistically credible (see fig. 2.4) between the weight build up by mother during the period of pregnancy and the concentration of PCB in milk, with the level of credibility p = 0.01. Correlation factor R^2 = 0.4403 corresponds with medium correlation.

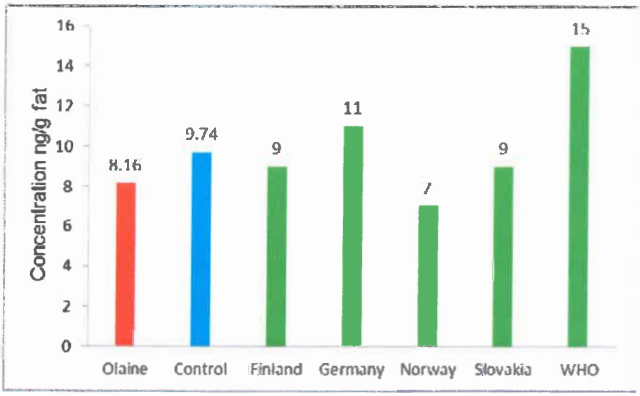


2.4. fig. Maternal weight gain during pregnancy correlation with PCB concentrations in milk

In our study fat was not determinated for each sample, but in other studies the average fat content in milk ranging from 2 - 5% (Chikuni et al, 1997; POPs in the Baltic, 2001; Soechitram et al, 2003; Weiss et al, 2003; Polder et al, 2009). The obtained results transformed from ng/ml of milk to ng/g of fat and compared to research conducted in other countries show that the POP levels correspond with the European mean values (fig. 2.5. and 2.6.).



2.5. fig. Marker-PCB concentration in breast milk samples in Latvia and other European countries (*Bąke* et al, 2007)



2.6. fig. PBDE concentration in breast milk samples in Latvia and other European countries (*Frederiksen* u.c., 2009)

2.4. Results of analysis of blood serum to detect the presence of POPs

The research covered 116 volunteers employed in metal-working and attendance of power equipment: 25 (21.6%) operators of power equipment (EG group) and 91 (78.4%) welders (ME group). All individuals involved in research were men. The results of descriptive statistical analysis of the research groups show that age, weight and height data of the subjects of research correspond with the general distribution and that they are comparable.

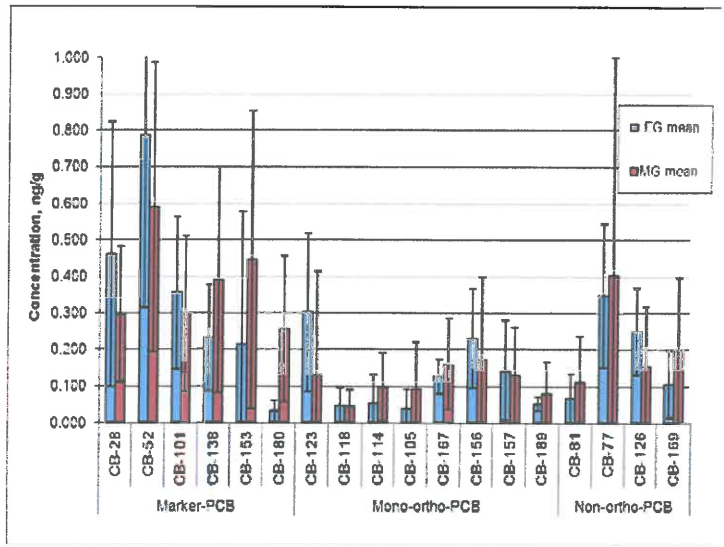
Three parallel gas chromatography measurements of the concentration of persistent organic pollutants were made on each analyzed blood serum sample. To facilitate comparison of the relevant POP concentrations in blood serum samples obtained from electricians and those of welders, the results were summarized in groups according to POP types: marker- PCB, mono-ortho-PCB, non-ortho- PCB and PBDE (table 2.4.). According to the results of measurements, the quantities of POPs detected by means of gas chromatography represent a highly wide concentration range: from level under the limit for detection of POPs to several ng/g of serum. Concentration of each POP in the sample was calculated according to the chromatographically measured quantity of POPs, subject to the volume of blood serum used for extraction and adjustment factor according to the internal standard. Half of the limit for detection of POPs (rather than zero value) was used as the minimum concentration for calculation of average values to avoid intentional decrease of values. The average POPs concentrations are given in 2.7. and 2.8. figures.

2.4. table

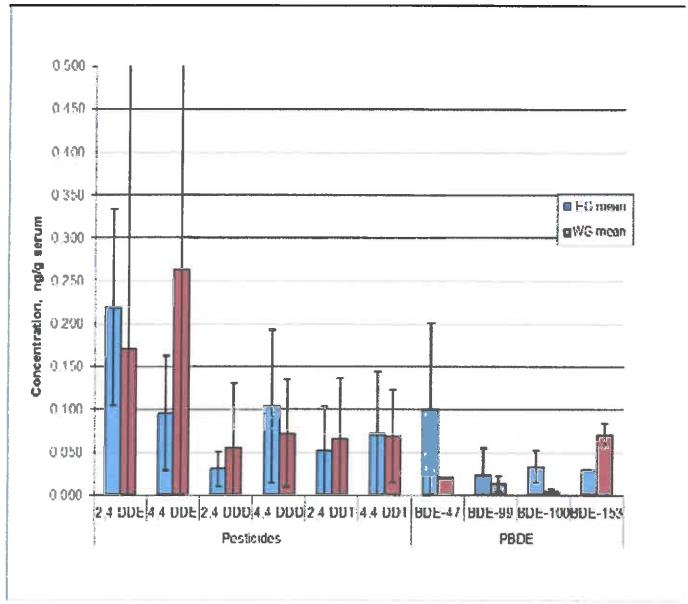
POPs average concentrations (ng / g), in electricians (EG) and welders (MG) blood serum (n = 116)

Sample	Marker-PCB sum	Mono-ortho-PCB sum	Non-ortho-PCB sum	Pesticides sum	PBDE sum
EG median	1.404	0.441	<0.010[a]	0.288	0.314
EG average	2.087	1.000	0.775	0,570	0.736
SD	0.973	0.510	0.295	0.227	0.364
min. conc.	0.144	0.063	<0.010[a]	0.026	<0.01[a]
maks. conc.	3.778	1.798	1.134	0.884	1.216
95% TI	0.944 – 1.962	0.267 – 0.912	<0.010[a] – 0.629	0.171 – 0.469	0.120 – 0.498
IQR ($Q_3 - Q_1$)	0.764 – 2.047	0.164 – 0.978	<0.010[a] – 0.745	0.154 – 0.508	0.082 – 0.528
EG TEQ pg/ml serum		0.249	26.144		
MG median	1.720	0.485	0.266	0.287	0.192
MG average	2.282	0.915	0.872	0.705	1.077
SD	1.075	0.514	0.486	0.713	0.807
min. conc.	0.553	0.026	<0.010[a]	0.033	<0.01[a]
maks. conc.	5.706	3.639	2.845	3.652	3.532
95% TI	1.468 – 1.931	0.403 – 0.578	0.194 – 0.354	0.234 – 0.353	0.128 – 0.260
IQR ($Q_3 - Q_1$)	1.233 – 2.253	0.279 – 0.737	0.111 – 0.557	0.169 – 0.529	0.010 – 0.477
MG TEQ pg/ml serum		0.232	17.711		
p-value	0.1007	0.9732	0.0369	0.6456	0.3193

[a]Below detection limits



2.7. fig. PCB medium concentration (γ average, ng/g serum) in electricians (blue) and welders (brown) group blood serum samples



2.8. fig. DDX and PBDE medium concentration (γ average, ng/g serum) in electricians (blue) and welders (brown) group blood serum samples

The presence of all twenty-eight POPs was identified in none of the analyzed blood serum samples. However, at least one pesticide, marker- and mono-ortho-PCB was identified in all samples. Non-ortho-PCBs were identified in blood serum samples of 52% of electricians and 97.8% of welders, and PBDEs were identified in 84% and 74.7% of the respective samples. HB-153 was the least frequently identified POP. It was not detected in any sample representing the group of electricians, and it was only detected in 10 samples representing the group of welders. According to HB-174, the mean arithmetical retrievability was 84.5 ± 14.5% and 84.6 ± 25.3% in the group of welders.

2.5. Analysis of working environment to detect the presence of POPs

Two power companies and three offices were selected for checking the eventual pollution of working places with POPs. Working environment at the power enterprises involves maintenance of powerful electric facilities (transformers, powerful condensers, electric engines, etc.). Samples of working environment were taken at locations where employees are exposed to eventual contact with oils and other PCB-containing materials. Samples of ambient air and working surface wash-offs were taken to ensure full assessment of working environment. Information about the concentration of POPs in the samples of working environment is summarized in Table 2.5.

Analysis of ambient air samples identified the presence of PCBs at one working place only: workshop for repairs of single-phase oil transformers were replacement of transformer oil was performed. On other occasions the concentration of POPs was below the detection level of the applied method. At least one of the below chlorinated market-PCBs was detected at each working place. Presence of marker-PCBs (HB-101 and HB-153) was detected in all samples.

2.5. table

POPs concentrations in the energy enterprises environment

Place	Sample type	Detected POP	Concentration	Standard Deviation
Repair area. Performs electrical equipment repairs	Air sample	Na[a]		
	Surface washings	CB-101	2.3 pg/m^2	0.5
		CB-77	2.1 pg/m^2	0.4
		CB-81	1.4 pg/m^2	0.3
		CB-153	0.9 pg/m^2	0.2
		CB-156	0.2 pg/m^2	0.1
Single-phase oil transformer repair area	Air sample	CB-101	550 pg/m^3	110
	Surface washings	CB-101	6.6 pg/m^2	1.3
		CB-77	6.6 pg/m^2	1.3
		CB-81	7.3 pg/m^2	1.5
		CB-153	4.5 pg/m^2	0.9
		CB-156	0.3 pg/m^2	0.1
		CB-167	0.3 pg/m^2	0.1
Electric department, carry out electric motors and other electric equipment repairs	Air sample	Na[a]		
	Surface washings	CB-52	5.6 pg/m^2	1.1
		CB-101	5.0 pg/m^2	1.0
		CB-77	5.6 pg/m^2	1.1
		CB-81	23.5 pg/m^2	4.7
		CB-153	2.2 pg/m^2	0.4
Welding station (energy enterprise)	Air sample	Na[a]		
	Surface washings	CB-28	2.6 pg/m^2	0.5
		CB-52	24.4 pg/m^2	4.9
		CB-101	6.4 pg/m^2	1.3
		CB-77	0.9 pg/m^2	0.2
		CB-81	1.3 pg/m^2	0.3
		CB-153	0.4 pg/m^2	0.1

[a]Was not detected in any samples

Assessment of working environment was conducted at three offices in Latvia to determine the eventual exposure of employees to POPs at their working places. Samples of ambient air were taken by two different methods as well as working surface wash-offs to ensure exhaustive assessment of working environment.

No presence of any PBDE or other POP was detected in any sampled taken on activated carbon. Gas chromatography analysis of filter extracts detected presence

of two to three PBDEs: BDE-47 (concentrations of 0.11 – 0.13 ng/g of dust), BDE-100 (concentrations of 0.03 ng/g of dust), and BDE-153 (concentrations of 0.12 – 0.76 ng/g of dust). No BDE-99 was detected in any sample. All extracts on cotton pads, on the turn, showed the presence of all four PBDEs: BDE-47 (concentration of 0.55 – 6.2 ng/g of dust), BDE-99 (concentration of 0.46 – 2.8 ng/g of dust), BDE-100 (concentration of 0.48 – 1.6 ng/g of dust), and BDE-153 (concentration of 29.2 – 37.5 ng/g of dust).

2.6. Analysis of food supplements to detect the presence of POPs

Five different food supplements (cod-liver oil) from different manufacturers were analyzed for the purpose of research. Analysis was aimed at detection of presence of twenty-eight POPs. The following cod-liver oil samples were analyzed:

- Of the company *Axellus A/S*, FUTURA Omega 3, Denmark;
- Of the company *Pharma Nord ApS*, Bio-Omega-3, Denmark;
- Of the company *Axellus A/S*, *Möller's* cod-liver oil tutti-frutti, Norway;
- Of the company *Silvanols SIA*, Silvanols cod-liver oil, Latvia;
- Of the company *Minskinterkaps*, *Eikonols*, Belorussia.

No presence of any POP was identified in analysis of the above-listed samples.

3. DISCUSSION

3.1. Comparison of methods applied to preparing of biological samples

Preparing of samples by ordinary extraction was approved as the first of methods. Such method was selected because it was relatively simple. The average retrievability obtained by such method made 23.9% of the theoretically possible value. It may be therefore concluded that retrievability is quite low, and it is not applicable to quantitative analysis. Development of the given approach was therefore found inexpedient because it provided the least result, compared to other methods, it was the most effort-consuming and involved consumption of large quantities of solvents.

Another method approved for preparing of samples was solid phase extraction where methyl alcohol was used as a solvent. The advantage of such method is application of factory-made chromatographic columns and equipment that enables good repetition ability on parallel samples. The quantities of solvents required for extraction were also notably smaller. The quantity of biological sample spent for analysis was only 5 ml, and this enables acceleration and simplification of sample-taking. Disadvantages include the particular care to be exercised by operator since even minor loss may lead to serious errors. Special attention has to be paid to purity of vessels.

The above-described method provides better results. The average retrievability of PCB – 174 amounted to 70.9% of the theoretically possible value, which is nearly three times higher compared to the previous described approach. Eventually, the achieved retrievability could even be higher if the milk sample was homogenized in ultrasound bath together with methyl alcohol, according to the approach described in literature (*Kočan* et al, 1994). Unfortunately, the results obtained by simple shaking turned out to be insufficient, and the analyzed subject stratified after some time.

The final method applied to preparing of samples was solid phase extraction with isopropanole as solvent. The solvent was selected notwithstanding that no method involving the use thereof was found in literature, because it was less harmful to human health, and also because it was readily available and caused no stratification of milk sample even without ultrasound treatment. According to Table 3.3, the latter approach provides the highest retrievability – 87.5%.

The obtained results lead to the conclusion that solid phase extraction with isopropanole as solvent is the most appropriate method for preparing biological samples for gas chromatography analysis in laboratory environment because it provides the highest retrievability of PCB – 174, namely 87.5%. The given method is simple, less effort-consuming and involves consumption of smaller quantities of solvent, compared to preparing of samples by simple extraction method. The fact that the applied solvent is less harmful to human health is also important.

3.2. Assessment of results obtained on milk samples

The results of survey conducted among young mothers lead to conclusion that Olaine target group and control group are comparable, and their characteristics show no significant differences. The survey covered 30 young mothers: 15 in Olaine group and 15 in control group, respectively.

The presence of marker–PCB in the samples indicates that the relevant compounds are identified in human body. Statistic processing of the obtained results enables the conclusion that in case of marker-PCB group compounds, the higher average concentrations were identified in Olaine group. No statistically credible difference between the two groups was identified in any marker-PCB, probably due to the small number of respondents and oscillation of the result plates from sample to sample.

According to the WHO data, the aggregate concentration of marker- PCB 6 in Central Europe varies from 200 to 400 ng/g (μg/kg) of milk fat (*Tuomisto* et al,

2011). The following aggregate concentration of the above-described compounds was identified in milk samples from the two groups: Olaine group – 2.28 ± 1.26 ng/g (ml) of milk, and control group – 1.69 ± 1.14 ng/g (ml) of milk. Recalculation of our results shows that the aggregate concentration of marker-PCB in the samples from Olaine group would amount to 114 ng/g of milk fat, and in control group to 84.5 ng/g of milk fat. Compared to the obtained results with data from other countries, all PCB values in Latvia turn out to be on the average European level; moreover, they correspond to the lowest European values (figure 3.7).

The presence of dioxin-type PCB group compounds (mono-, ortho-, and non-ortho) is discussed separately. Such compounds are similar to dioxins by their structure, and their potential impact on health is commonly assessed by toxicity equivalent (TEQ) determined on the basis of toxicity factors (TEF) of each individual compound. The aggregate toxicity equivalent (TEQ) represents the potential toxicity of the compound, and it is among the key parameters considered in assessment of risk to human health. It is used to transform individual substances (dioxins, dioxin-type PCBs and Furans) into dioxin which is more toxic and better studied – 2,3,7,8-tetrachlorodibensodioxin (*The Air Toxics Hot Spots Program Guidance Manual for Preparation of Health Risk*, 2012; *WHO*, 1998).

Statistically credible difference ($p<0.05$) in the group of mono-ortho-PCB compound group in case of HB-105 and mono-ortho-PCB aggregate concentration. Difference near to statistical credibility between the groups was also detected in case of HB-118 ($p=0.0649$) and HB-157 ($p=0.0712$). In case of the latter PCB group, higher average concentration was also detected in the samples from Olaine group, with the exception of HB-123 identified in higher concentration in the samples from control group. Therefore, the aggregate toxicity equivalent (TEQ) was higher in the samples from Olaine group (0.428 pg/ml of milk), compared to the control group (0.174 pg/ml of milk).

In case of non-ortho-PCB, as well as marker-PCB, no statistically credible difference ($p>0.05$) was identified among the groups in any of the compounds. As regards such compounds, higher concentrations were detected in Olaine group in case of aggregate concentration of HB-81, HB-169, and non-ortho -PCB, while the control group showed higher concentrations of HB-77 and HB-126. Since HB-126 is the most toxic PCB, the aggregate toxicity equivalent (TEQ) in control group (9.569 pg/ml of milk) was higher, compared to the Olaine group (7.730 pg/ml of milk), however no statistically credible difference was identified between the two groups ($p=0.8519$).

According to the definition of World Health Organization, the aggregate conditional harmless TEQ in human milk is 15 pg/g (*WHO*, 1998). In case of our research, the aggregate TEQ was 8.158 pg/g in case of Olaine group and 9.743 pg/g in control group, which is below the level recommended by WHO.

The following results have been obtained in similar research conducted during the period from 2002 to 2004:

- Finland (Helsinki) – 9 pg/g;
- Germany (Berlin) – 11 pg/g;
- Norway (urban population) – 7 pg/g;
- Slovakia (urban population) – 9 pg/g (*Bąkę* et al, 2007).

It may be concluded from the foregoing that pollution and toxicity of human milk in Latvia corresponds to the average European level, and that no increased risk to newborn children exists, compared to other countries of Europe.

Food is the most common source of POPs in human body; in most countries, food makes up to 90% of all POP intakes (cod-liver oil, milk, fatty fish). Other intakes include contaminated water, air and skin contact, and irrelevant conditions. It is recommended to replace fatty food products to less fatty, such as lean cheese and vegetable oils that correspond to healthy food habits. Spanish scientists have discovered that the period during which intake of PCBs with food has taken place is beyond determination; it may therefore true that concentration of PCB in milk

may also be influenced by body constitution, metabolism and individual absorption capacities. Measuring intake by food may be therefore erroneous since it probably is just a confounding variable (*Chikuni* et al, 1997; *POPs in the Baltic*, 2001; *Soechitram* et al, 2003; *Weiss* et al, 2003; *Polder* et al, 2009). The amount of PCBs in human milk may also vary from season to season. The level of PCBs in human milk is higher in summer. This may be explained by the fact that women tend to accelerated loss of weight in summer, and therefore the content of fat in human body decreases more intensively than in winter (*Soechitram* et al, 2003). In our research, we have managed to identify correlation between the frequency of fatty food intake and concentration of PCBs in milk (figure 3.5). It may be due to the small number of respondents and high diversity of results among mothers.

In our research, only primiparae were selected as milk donors because the first child is exposed to the highest concentrations of PCBs since the concentrations of PCB in the milk of primiparae is higher compared to mothers of several children (*POPs in the Baltic*, 2001; *Polder* et al, 2009).

Anthropological parameters (average age, average height) of mothers were highly similar in both groups, while the average weight was higher in case of mothers from Olaine group by 6 – 7 kg, compared to the control group. The key anthropologic factor in case of mothers involved in our research was the gain in weight during the period of pregnancy, because it presents the only statistically credible ($p = 0.01$) correlation of PCB levels ($R^2=0.4403$) (Figure 3.6). Similar correlations are also detected by authors of other works; in Germany, for example, it is expressly recommended to avoid loss of weight immediately after childbirth because additional fat is released during breast-feeding, and therefore higher concentrations of PCBs are transferred to the child (*Schlaud* et al, 1995).

No correlation was found in our research between the birth weight of child and concentration of PCB in milk. It may be explained by the small number of respondents. Researchers in Germany and Sweden, for example, have detected that

PCB-exposed children have lower birth weight, compared to the non-exposed ones (*POPs in the Baltic*, 2001).

Comparison of the two groups shows that PBDEs are much less frequently found in the control group. The aggregate concentration in case of Olaine group (0.069 ng/ml of milk) was therefore higher that in control group (0.0313). The same is true in comparison of the average, minimum and maximum concentrations of individual representatives of PBDEs. The results lead to conclusion that people in urbanized environments are more exposed to the impact of PBDEs than population in rural regions. Research conducted in Norway also displays the relation identified between the location of residence and the level of PBDEs in human milk (*Thomsen* et al, 2010). Similar to other research conducted in Europe, our research also reveals the highest concentrations of BDE-47 (*Jaraczewska* et al., 2010; *Lignell* et al., 2010; *Thomsen* et al, 2010). The results of our research denominated in the amount of fat lead to conclusion that the average aggregate concentration of PBDEs in Olaine group is 3.45 ng/g of fat, while the average value in control group is 1.57 ng/g of fat. Comparison of the obtained results to data from other countries shows that the PBDE values in Latvia are on the average European level; moreover, the results obtained from control group correspond with the lowest values in Europe (Figure 3.8).

Based on the data of conducted research and comparison of the obtained results with reference data lead to conclusion that human milk in Latvia corresponds to the lowest European and global levels by all analyzed POPs, and therefore no increased risk to children's health exists, compared to other countries.

3.3. Assessment of the results of blood serum samples

The presence of POPs was detected in bodies of the representatives of risk group – those employed in metal-working and operation of powerful electrical equipment. Just like in our research, authors of other studies also point out to the

fact that concentrations of POPs in blood serum vary in wide range. According to reference data, food is the basic source of intake of POPs, in particular food with high fat content (*Meneses* et al., 1999; *Agency for Toxic Substances and Disease Registry*, 2000; *Agency for Toxic Substances and Disease Registry*, 2004; *Schecter* et al, 2010; *Frederiksen* et al, 2010).

In our research, no significant differences in eating habits were identified; the respondents included a vegetarian, yet it had no significant effect on the results; the other respondents consumed mixed food, and it may be therefore concluded that eating habits present no essential confounding variable.

A number of representative studies involving large numbers of respondents and conducted during several years are mentioned in literature. A study conducted in the US, for example, summarizing data about the levels of PCBs in the population of the US, includes 45 published studies conducted during 41 years from 1963 to 2003 and covering 16914 respondents (*Hopf* et al, 2009). Results of the above-described study enable conclusion that the aggregate concentration of PCBs in blood serum trends to decrease in course of time. This is due to the fact that the US started reducing the application of PCBs from 70s of the 20th century. Studies conducted from 2000 are used to obtain the average concentrations to avoid increased reference values. The average values have been collected from eighteen studies in total. The average concentration obtained during the US study equals to 3.76 ± 3.19 ppb (ng/g), while in our research it is 3.86 ± 3.22 ng/g in the group of electricians and 4.069 ± 3.10 ng/g in the group of welders respectively. Our results are therefore comparable to the average PCB levels in the US population.

According to the results of studies, concentration of PCBs in blood depends on the respondent's age. For example, in the study conducted by *Jackson* (2010), the aggregate concentrations of PCBs in blood serum of mothers (n = 44) was 4.18 ng/g, while in 24 m.o. children (N = 17) it was 0.88 ng/g of serum. We did not

manage to identify any correlation between the POP levels in blood serum and the respondents' age or duration of their employment.

All six of the identifiable marker-PCBs have only been detected in one sample representing the group of electricians, were found while in the group of welders they are present in 41 samples (45%). Statistically credible difference between the groups has been established in case of CB-28, CB-138, CB-153, and CB-180.

In case of samples representing the group of electricians, higher concentrations of lower chlorinated marker-PCBs, while higher chlorinated marker-PCBs were detected in the group of welders. The aggregate average concentrations of CB-28, CB-52, and CB-101 were higher in the group of electricians where it was equal to 1.604 ng/g of serum, while in the group of welders it amounted to 1.187 ng/g of serum. Higher concentrations of the concerned substances in blood serum may serve as evidence to pollution of working place with PCBs or contact of employees with PCB-containing substances and products (*Broding* et al, 2008;. *Broding* et al, 2007; *Liebl* et al, 2004).

The aggregate toxicity equivalent factor (TEQ) of mono-ortho and non-ortho polychlorinated biphenyls amounted to 26.39 pg/g of serum in the group of electricians, while in the group of welders it was 17.94 pg/g of serum. Statistically credible difference ($p<0.05$) in compounds of the given groups was established in the aggregate of CB-105, CB-118, CB-167, CB-189, CB-77, CB-169, and non-ortho-PCBs. It shows that dioxin-type PCBs are present in the blood of electricians with higher statistic credibility than in case of the group of welders.

Comparison of our results with those obtained from research in other countries shows that the former are higher. In Swedish study, for example, analysis of blood samples of pregnant women ($n = 325$) showed TEQ equal to 3.21 pg/g (*Glynn* et al, 2007). Similar results were obtained by UK researchers (*Thomas* et al, 2006). They analyzed blood serum samples taken from 154 volunteers to detect halogen-organic compounds and established TEQ equal to 3.70 ng/g. The results obtained by researchers from Thailand were higher (*Lung* et al, 2005). They analyzed blood

serum samples taken from people who had been consuming PCB-contaminated oil in 1979 (n = 414) and from children (n = 21) born after the accident. The average TEQ value in the group of children was 32.0 ± 29 pg/g, while the average TEQ in adult group was 84 ± 10 pg/g. Higher TEQ in the groups of welders and electricians, compared to the results of studies conducted in Sweden and the UK may be explained by the fact that the latter studies only involved analysis of mono-ortho-PCBs in serum. If the results are only compared by mono-ortho-PCBs, which in our case is 0.249 pg/g in the group of electricians and 0.232 pg/g in the group of welders, the welders and electricians in Latvia are not exposed to higher risk to their health due to dioxin-type PCBs than respondents of the two above-mentioned studies.

According to reference data, major of the DDX concentration forms from p, p'-DDE (*Walizewski* et al, 1999; *Tomass* et al, 2006;. *Glynn* et al, 2007; *Draper* et al, 2007). In our research, the concentration was estimated at 0.095 ng/g of serum in the case of electricians, and 0.263 ng/g in the case of welders. Comparison of our results with those obtained from research in other countries leads to conclusion that the aggregate concentration of both p, p'-DDE and DDT was lower in our research. For example, concentration of p, p'-DDE in blood serum of inhabitants of Veracrusa, Mexico, was 14.5 ± 28.0 ng/g of serum, while the total DDT was – 16.4 ± 30.8 ng/ml (*Walizewski* u.c., 1999), and in the study conducted in the US concentrations of p, p'-DDE oscillated within the range of 0.17 to 8.9 ng/ml of serum (*Draper* et al, 2007). Smaller concentrations of DDT in blood serum samples taken from employees in Latvia may be explained by use of smaller quantities of the given substances in Latvia.

Polybrominated diphenylethers PBDEs present a relatively new issue of POPs in Latvia. Reference data show that concentration of PBDEs in biologic environments of human and other bodies trend to increase in course of time (*Noren* and *Meironyte*, 2000; *She* et al, 2002; *Toms* et al, 2006). This is due to ample consumption of products that contain such compounds. Like other POPs,

concentrations of PBDEs in our research vary in wide range: from concentration below the detection limit of the applied method to 1.216 ng/g of serum in the group of electricians and 3.532 ng/g of serum in the case of welders. Similar situation could also be observed in other studies. In Australia, for example, concentration ranged from 6.4 ng/g of fat to 80.0 ng/g of fat, and in Netherlands – from 0.010 ng/g of serum to 1.012 ng/g of serum (*Toms* et al, 2006; *Peters*, 2004). The Australian study includes analysis of 85 combined samples taken from 8132 respondents. The results are expressed in ng/g of fat, with possible transformation into ng/g of serum because of the stated data about the average contents of fat in samples. The average aggregate concentrations of PBDEs in the group of electricians made 0.736 ng/g of serum and 1.077 ng/g of serum in the group of welders, compared to the Australian study where aggregate concentration of PBDEs in adults (age of group > 16 years) in 2002/ 2003 was 0.083 ng/g of serum, and in 2004/2005 it was 0.100 ng/g of serum.

Compared to the group of pregnant women in Californian study where the aggregate concentration of PBDEs was 1.4 and 1.9 ng/ml of serum, respectively, the concentration of PBDEs obtained in our research was the lowest (*Toms* et al, 2006; *Draper* et al, 2007). Similar to other studies, the most frequently identified and the highest concentrations were presented by tetra BDE-47 (*Sjödin* et al, 1999; *Tomass* et al, 2006; *Draper* et al, 2007). In our research, the average concentrations were 0.344 ng/g of serum in the group of electricians and 0.551 ng/g in the group of welders, respectively; while in Australian study the relevant value was 0.027 ng/g of serum.

The key factors that can affect health condition of an individual include smoking. We did not manage to identify relation between smoking habits and the levels of POPs in blood serum of employees. No statistically credible difference ($p>0.05$) between the groups was identified in any POP sample.

3.4. Assessment of the results of samples taken from working environment

The results of our research show that POPs are present in different working environments. The research included study of three offices and two power enterprises, and the presence of POPs was identified in all occasions.

Three sample-taking approaches were approved for detection of the presence of POPs:

- Collecting POP vapor from ambient air on activated carbon;
- Collecting dust from ambient air on filters;
- Collecting wash-offs from working surface on medicinal cotton pads.

The obtained results show that the approach that involves collecting of vapor is the least efficient one because no presence of POPs has been detected in activated carbon samples. The best results have been obtained from collecting wash-offs from working surfaces. The highest quantities of POPs and numbers per sample have been identified in the samples obtained by this approach.

The presence of PBDEs was detected in all offices, both in dust in the ambient air and on surface wash-offs. It may be therefore concluded that the office equipment operated in Latvian offices contains PBDEs, and that employees are exposed to the risk of impact of such substances.

Analysis of working environment to detect the presence of POPs in power enterprises revealed presence of PCBs in all working places. Marker-PCBs were mainly identified. The presence of CB-101 and CB-153 was established in all working places. It was also identified that the relevant compounds are present not only in working places that involve maintenance and repairs of power equipment but also in welding workshop. Consequently, the compounds trend to spread on the entire territory of the enterprise, and all employees of the enterprise are exposed, to certain extent, to the risk of impact of the given compounds.

Summarized results of analysis of the working environment lead to conclusion that POPs present an urgent issue and that working environment is likely to influence the level thereof in the bodies of employed individuals.

3.5. Assessment of the results of cod-liver oil samples

Cod-liver oil may present an essential source of contamination of body with POPs (*Meneses* et al., 1999; *Agency for Toxic Substances and Disease Registry*, 2000; *Agency for Toxic Substances and Disease Registry*, 2004; *Schecter* et al, 2010; *Frederiksen* et al, 2010). Medicine professionals often recommend this food supplements to pregnant women and other patients.

The research involved analysis of five different cod-liver oil products available from pharmacies in Latvia, intended for people of all ages and representing different price groups. None of the 28 analyzed POPs was detected in any of the analyzed samples. It may be concluded that POPs have been qualitatively removed from cod-liver oil available from pharmacies in Latvia, and that they present no risk to the users' health.

CONCLUSIONS

Purification of biological samples with solid phase extraction where isoprophanol is used as the solvent and the selected gas chromatography parameters enable qualitative and quantitative analysis of environmental and biological samples to detect the presence of POPs (the approach ensures sufficient selectivity and sensitivity).

The selected target and control groups are representative, comparable and reflect the risk caused by POPs in the studied groups.

The weight build up by mothers during the period of pregnancy correlates with the level of PCBs in milk (R^2=0.4403); no correlation has been identified, however, between PCBs and the frequency of fat food consumption. The POP levels identified in biological environments of young mothers, electricians and welders correspond to the average European and global levels.

The POPs detected in biological samples taken from employees confirm that they are presented in working environment.

Cod-liver oil (food supplement) dos not present an essential source of POPs.

The proposed hypothesis regarding insufficient study of POP levels in population and application of appropriate methods to analysis of various biological samples has been confirmed.

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