



## Levels of Selected Persistent Organic Pollutants in Human Milk Samples from Bosnia and Herzegovina

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**Abstract:** One of the greatest challenges of today is environmental pollution, which leads to serious threats to human health and the entire ecosystem. Due to their high persistence and low biodegradability, polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs), as the main categories of environmental pollutants of anthropogenic origin, remain present in the environment for a long period of time, continuously circulating between air, water and soil, posing a permanent risk to public health. Bosnia and Herzegovina has not previously conducted studies on the presence of environmental pollutants in breast milk, the results of which could contribute to more effective public health protection and serve as a basis for strategic planning aimed at reducing exposure to environmental pollutants. For this reason, the aim of this study was to determine the levels of selected persistent organic pollutants (PCBs and OCPs) in breast milk of lactating women (N=67) from Bosnia and Herzegovina (Federation of Bosnia and Herzegovina, Republika Srpska and Brčko District). The results presented here are the first of their kind for the territory of our country. The average values of quantified concentrations of PCB (17 congeners) and OCP (8 compounds) ranged from 1.03-162.29 ng/g l.w. and from 0.27 to 14.64 ng/g l.w., respectively. PCB congeners 52, 77, 126, and 169 were detected at higher levels compared to those reported in some of the European and Asian countries. In Brčko District and Republika Srpska, higher values were found compared to the Federation of Bosnia and Herzegovina. The measured concentrations of selected persistent pollutants in breast milk samples collected in Bosnia and Herzegovina mostly correspond to the literature data from similar studies conducted in the countries of the region and Europe.

## INTRODUCTION

Environmental pollutants can arise due to numerous processes of natural and anthropogenic origin. Pollutants of anthropogenic origin include three main categories of persistent organic pollutants (POPs): industrial chemicals (e.g., polychlorinated biphenyls, PCBs), pesticides (e.g., organochlorinated pesticides, OCPs), and unintentionally produced co-products of industrial processes and combustion (dioxins and furans). The basic characteristics of POPs such as environmental persistence, semi-volatility, as well as lipophilicity allow them to accumulate over time as well as biomagnify through the food chain, resulting in their appearance in the organisms of humans and animals that are at the top of the food chain. Until the 1970s, polychlorinated biphenyls (PCBs) were widely used in various industries, in electrical equipment, transformers and capacitors, plastics, paints and pesticides. OCPs have been used in agriculture as insecticides, acaricides and fumigants in various crops, and after World

War II they played a key role in the control of malaria and typhoid fever (Aktar et al., 2009; EI-Shahawi et al., 2010; Zhang et al., 2006).

Although their production has long been banned internationally, these compounds may still be released into the environment due to illegal or improper disposal of PCB-containing waste as well as continued use of OCP in agriculture. Due to their low biodegradability, they remain in the environment for a long time, circulating between air, water and land, and continue to raise major public health concerns. (ATSDR, 2000). The human body can be exposed to PCBs and OCP by respiratory, oral or dermal routes. (Fitzgerald et al., 2004; Freels et al., 2007).

Many studies show that exposure to PCBs and OCPs can be associated with various health problems affecting the nervous, immune, endocrine, and reproductive systems, as well as result in cancerous changes. (Longnecker, Rogan and Lucier, 1997; Ma and Sassoon, 2006; Vafeiadi et al., 2017; Rissato et al., 2006; Olisah et al., 2020). PCBs are also recognized as endocrine disruptors, because they can

mimic the action of endogenous hormones by binding to hormone receptors or blocking them, thus altering the normal function of the endocrine system. (Ma and Sassoon, 2006; Wang *et al.*, 2006).

In addition, maternal contamination may serve as a source of fetal exposure prenatally and, in infants, postnatally through breast milk. (Darnerud *et al.*, 2010; Turrio-Baldassarri *et al.*, 2007) It is believed that infant exposure to PCBs through ingestion of contaminated breast milk may affect their growth and neurological development. (Dekoning and Karmaus, 2000; Meeker, 2012; Todaka *et al.*, 2010). Breast milk is considered an ideal matrix for biomonitoring due to its easy availability, non-invasive sampling method, and its high lipid content, which facilitates the accumulation of lipophilic pollutants.

Although the use of persistent pollutants in BiH and neighboring countries was banned 40 years ago or strictly regulated in recent decades, their residues are still detected in water, air, soil and food. In BiH, PCBs were used in industry and their presence was discovered in old electrical equipment and closed systems such as transformers, capacitors and switches, as well as in barrels used to store spent transformer oils. Between 2008 to 2013, according to the data of the Agency for Statistics of BiH, a certain amount of mixtures and preparations containing PCBs, polychlorinated terphenyls (PCT) or polybrominated biphenyls (PBB) were imported. (NIP, BiH) Investigations of persistent organic pollutants levels in the Neretva River revealed the presence of dichlorodiphenyltrichloroethane (DDT) and its metabolites, as well as polybrominated diphenyl ether (PBDE) congeners 47 and 99. Although the concentrations of the POPs tested in the aforementioned study were below local standards for environmental protection and human water consumption, the levels of dieldrin and total PCBs were above Environmental Protection Agency (EPA) standards for human consumption. (Djedjibegovic *et al.*, 2010) Also, the National Implementation Plan for BiH reports incident situations of unintentional PCB releases from capacitors into the environment, as well as improper storage of old PCB-containing equipment.

All of the above highlights the importance and need for continuous monitoring of persistent organic pollutants in both the environment and food, as the primary source of exposure of the general population to these pollutants.

Long-term and repeated studies of POPs in breast milk, conducted both in the region (Krauthacker, 2000; Runkel, 2021; Vukavić *et al.*, 2008) and globally, provide data useful for monitoring spatial and temporal trends of pollutant load in the general population, which is also one of the indicators of environmental burden. In general, these studies reported on a temporal decrease in POPs concentrations attributed to a decrease in their overall emission, but also certain geographical variations, which highlight the importance of sampling sites distribution within the monitoring process. Bosnia and Herzegovina is the only country in the region that does not have data on the content of persistent organic pollutants in breast milk, which indicates the exceptional importance of this research for the entire country.

Accordingly, the aim of this study was to determine the levels of selected persistent organic pollutants (PCBs and

OCPs) in breast milk of lactating women from Bosnia and Herzegovina.

## EXPERIMENTAL

### *Materials and methods*

#### *Research Protocol and Design*

The study was designed as a cross-sectional study in which breast milk samples were collected in accordance with the guidelines of the World Health Organization (WHO) (UNEP, 2017) taking into account regional representation to ensure a nationally representative sample. The research was approved by The Ethics Committee of the University of Sarajevo-Faculty of Pharmacy for Experimental Research in the Scientific Projects (No. 0101-1267/20 of March 13, 2020). The recruitment of potential breast milk donors was carried out across Bosnia and Herzegovina (BiH) in cooperation with private and public institutions of primary and secondary health care, through social media networks, as well as by distributing information leaflets about the study. Participation in this study was entirely voluntary. A total of 130 mothers were recruited as potential breast milk donors. The main inclusion criteria were that the mother was under 40 years of age, with a normal and healthy pregnancy, with a ten-year residence in the study area, and that a breast milk sample was collected between the third and eighth week postpartum. The exclusion criteria were the mother's (child's) potential specific exposure which could significantly deviate from the national average, as well as refusal to sign the informed consent form.

Breast milk samples were collected in glass containers, previously treated at 450°C and rinsed with an organic solvent. A minimum of 50 ml of milk sample was collected manually. Milk samples were stored at 4°C for up to 72 hours and then frozen at -20°C until analysis. Before laboratory analysis, frozen samples were heated to 40°C and homogenized to ensure complete fat dissolution and distribution across the sample.

Socio-demographic characteristics of the donors such as age, level of education, occupation, place of residence, cigarette consumption, etc., as well as height and pre-pregnancy weight were collected through a questionnaire. Statistical significance of differences among groups was tested by the Mann-Whitney U test, while correlations between individual sociodemographic variables and POPs concentrations were assessed by Spearman's rank correlation. p-values lower than 0.05 were considered statistically significant.

#### *Chemical Analysis*

Chemicals and reagents hexane (from Honeywell, Germany), 2-propanol (all analytical grade), amyl alcohol (sp. gr. 0.185) and tetrabutylammonium sulphate (p.a.) were purchased from Sigma-Aldrich (Seelze, Germany). Sodium sulphite (p.a.) was purchased from Kemika (Zagreb, Croatia). Sulphuric acid (98%, p.a.) was purchased from Merck (Darmstadt, Germany) and sulphuric acid (90-91% p.a.) from Grammol, Croatia. PCB standards were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany). OCP standards were purchased from Sigma-Aldrich (Germany). Nitrogen 5.0 was supplied from Messer (Sarajevo, Bosnia and

Herzegovina). Ultrapure water (>18mΩ) was supplied by Arrium® mini Lab Water System (Goettingen Germany). For fat content determination and its isolation from breast milk samples we used Gerber's method (Sabadoš D., Rajšić B., 1964), as a quick and simple method for determining the fat content in milk and dairy products of animal origin. After homogenizing the sample, the butyrometer was filled first with 10 ml of 90% sulfuric acid, then with 11 ml of breast milk and finally with 1 ml of amyl alcohol. After gentle mixing, the butyrometers were placed in a water bath for five minutes at 65°C. Then centrifugation was performed at 1100 rpm for 5 minutes, and repeated twice. After centrifugation, the butyrometers were again placed in the water bath for 5 minutes at 65°C. Then the percentage of isolated fat was read on the butyrometer scale. The entire fat contents were transferred into tubes

with a conical bottom and ground stopper and treated with 2 ml of hexane. The tubes were vortexed for 2 minutes, placed in an ultrasonic water bath for 15 minutes and then on a shaker for 30 minutes at 80 rpm. The hexane extract was then subjected to a purification procedure with concentrated sulfuric acid, as described by Djedjibegovic et al. (2015)

#### Quality Control/Assurance

Quality control was carried out by analyzing a spiked breast milk sample as well as one laboratory blank of reagents for each batch of samples. All samples were analyzed in duplicate, and the content of the tested analytes was presented as an average. The values of the analytical parameters of the method used for PCB congeners and OCP components are shown in Table 1.

Table 1. Values of analytical parameters for test analytes (PCB and OCP)

Analytes of interest	R2	LD (ng/g l.w.)	LQ (ng/g l.w.)	Average RF (%)	RSD (%)
<b>α- BHC</b>	0.9998	0.22	0.665	89.00	4.73
<b>β- BHC</b>	0.9997	1.167	3.535	80.26	5.01
<b>γ- BHC</b>	0.9996	0.339	1.027	82.51	5.08
<b>HEPTACHLOR</b>	0.9958	2.324	7.042	105.56	9.04
<b>ALDRIN</b>	0.998	0.321	0.972	20.19	4.46
<b>HEPTACHLORE EPOXIDE ISOMER B</b>	0.9982	2.2	6.667	49.48	5.26
<b>2,4' DDD</b>	0.9993	2.704	8.193	70.18	3.73
<b>DIELDRIN</b>	0.9987	2.849	8.635	151.72	3.74
<b>2,4'-DDT*</b>	0.9988	5.134	15.556	/	/
<b>4,4'-DDT</b>	0.9997	3.025	9.166	3.25	5.46
<b>PCB 28</b>	0.9984	0.281	0.851	131.843	5.13
<b>PCB 52</b>	0.9995	0.322	0.976	108.324	4.69
<b>PCB 101</b>	0.9981	0.299	0.907	98.603	5.13
<b>PCB 153</b>	0.9993	0.486	1.473	102.964	4.55
<b>PCB 138*</b>	0.998	0.388	1.176	/	/
<b>PCB 180</b>	0.9981	0.3	0.908	116.609	4.07
<b>PCB 209</b>	0.9973	0.363	1.101	87.297	4.53
<b>PCB 30</b>	0.9987	0.315	0.956	ND**	24.05
<b>PCB 123</b>	0.9985	3.412	10.34	ND**	12.5
<b>PCB 156</b>	0.9995	0.315	0.956	ND**	5.2
<b>PCB 77</b>	0.9984	2.781	8.426	ND**	11
<b>PCB 105</b>	0.9991	0.349	1.058	ND**	24.8
<b>PCB 118</b>	0.9967	1.681	5.093	ND**	3.3
<b>PCB 126</b>	0.9996	0.361	1.095	ND**	3.7
<b>PCB 157</b>	0.9994	0.34	1.031	ND**	6.5
<b>PCB 167</b>	0.9963	1.767	5.355	ND**	6.86
<b>PCB 169</b>	0.9948	6.322	19.157	ND**	13.6
<b>PCB 204</b>	0.999	0.576	1.746	ND**	7.4

\* 2,4'-DDT and PCB 138 co-eluted so quantification was not possible

\*\*ND – not determined (RFs were determined only for indicator PCBs mix shown in the table)

#### Determination of selected organic pollutants (PCBs and OCPs)

Determination of PCB and OCP compounds were performed by gas chromatography with electron capture detector (GC/ECD) using Agilent Technologies (Santa Clara, USA) 7890A GC as described in Djedjibegovic et al (2010). Briefly, the Agilent DB-5 column (60 m × 0.25 mm × 0.25 μm) with a stationary phase of 5% phenyl polysiloxane was used. Column flow rate was 1 mL min<sup>-1</sup>. The oven temperature program was the following: 90°C (2 min), 10°C/min to 180°C, then 2°C/min to 270°C and finally 20°C/min to 290°C (20 min). The detector temperature was 285°C and injection volume 1 μL. The inlet was operated in splitless mode.

#### Statistical analysis

Statistical analysis was performed using the SPSS Statistical Package, version 2023. Descriptive statistics, including mean, standard deviation (SD), and relative standard deviation (RSD), were calculated to evaluate the

distribution of target analytes in the analyzed milk samples. Since the data were not normally distributed according to the Shapiro-Wilk test, nonparametric statistical tests were applied. Statistical significance was considered at  $p < 0.05$ .

## RESULTS AND DISCUSSION

A total of 67 breast milk samples were collected from the area of 2 entities of BiH, Federation of BiH (FBiH, n=37) and Republika Srpska (RS, n=28), and Brčko District (BD, n=2). Out of a total of 67 donors who completed the research, 66 of them filled out a questionnaire with sociodemographic characteristics. Table 2 shows the socio-demographic characteristics of breastfeeding mothers.

Table 2 Sociodemographic characteristics of lactating mothers

	Options	Total n	% of all respondents	FBiH	RS	BD
				n (%)*	n (%)*	n (%)*
Place of residence	Urban	52	78.79	28 (75.68)	22 (78.57)	2
	Rural	14	21.21	8 (21.62)	6 (21.43)	/
Parity	primiparae	38	57.58	25 (67.57)	11 (39.29)	2
	multiparae	28	42.42	11 (29.73)	17 (60.71)	/
Level of education	Basic	2	3.03	/	2 (7.14)	/
	Secondary	18	27.27	10 (27.03)	8 (28.57)	/
	High	46	69.70	26 (70.27)	18 (64.29)	2
Smoking status	Non-smokers	46	69.70	23 (62.16)	21 (75.00)	2
	Smokers	20	30.30	13 (35.14)	7 (25.00)	/
		<b>n (mean)</b>	<b>% of all respondents</b>	<b>n (mean)</b>	<b>n (mean)</b>	<b>n (mean)</b>
BMI	<18.5	4 (18.32)	6.06	1 (18.42)	2 (18.38)	1 (18.07)
	18.5 – 24.9	47 (22.11)	71.21	29 (22.11)	17 (22.12)	1 (21.77)
	25.0 – 30.0	13 (26.74)	19.70	5 (25.95)	8 (27.23)	/
	>30.0	2 (33.59)	3.03	1 (35.29)	1 (31.89)	/

n (%)\* - the number and percentage of respondents in relation to the number of respondents per entity/district

The percentage of fat isolated in breast milk samples ranged from 1.3% to 6.7% with an average value of 3.51%, which is consistent with the values recorded in the literature.

#### Levels of selected PCBs in human milk samples

In the analyzed breast milk samples, the average concentrations of the following PCB congeners were determined: 28, 52, 101, 153, 180, 209, 30, 77, 123, 118, 105, 126, 167, 156, 157, 204 and 169 and expressed in ng/g

l.w. The indicator congener PCB 138 could not be quantified due to the same retention time as 2,4'DDT from the OCP group. The values of relative standard deviations were below 30% in most samples, which is acceptable according to the relevant guidelines (AOAC (2016), European Commission, 2021) and indicate good reproducibility of the method. The exception was a certain number of samples with concentrations close to the limits of detection and quantification with elevated RSD values,

which is expected when measured at very low concentrations.

Table 3 shows the results of descriptive statistics for selected PCB congeners (28, 52, 101, 153, 180, 209, 30, 77, 123, 118, 105, 126, 167, 156, 157, 204 and 169)

Table 3 Descriptive statistics for selected PCB Congeners

	N	Average	SE	Median	SD	Variance	Min*	Max
<b>PCB 28</b>	34	17.74	5.15	10.05	30.02	901.29	0.43	163.87
<b>PCB 52</b>	66	162.29	12.67	136.78	102.92	10591.79	16.4	495.77
<b>PCB 101</b>	45	9.17	1.35	6.11	9.07	82.35	0.15	49.78
<b>PCB 153</b>	58	3.52	0.37	3.05	2.79	7.77	0.25	15.7
<b>PCB 180</b>	63	3.68	0.43	2.85	3.44	11.83	0.46	23.96
<b>PCB 209</b>	16	6.97	2.78	2.67	11.11	123.5	0.19	40.4
<b>PCB30</b>	65	21.16	1.89	17.11	15.22	231.77	6.11	103.8
<b>PCB77</b>	22	7.32	1.76	5.72	8.26	68.27	1.89	38.79
<b>PCB123</b>	1	1.74	/	/	/	/	/	/
<b>PCB118</b>	42	4.27	0.59	2.6	3.8	14.41	0.86	16.78
<b>PCB105</b>	42	1.64	0.39	0.54	2.53	6.4	0.18	11.97
<b>PCB126</b>	49	3.21	0.28	2.87	1.96	3.86	0.18	10.96
<b>PCB167</b>	29	82.93	24.39	33.37	131.34	17250.66	0.9	575.21
<b>PCB156</b>	23	1.03	0.17	1.01	0.79	0.63	0.16	3.01
<b>PCB157</b>	27	2.5	0.35	2.1	1.82	3.32	0.17	8.29
<b>PCB204</b>	9	3.52	1.61	2.37	4.84	23.45	0.89	16.07
<b>PCB169</b>	31	18.63	2.73	9.77	15.18	230.54	3.22	60.96

\* minimal concentrations for all congeners except PCB 52, PCB 30 where lower than LQ

PCB congeners 52, 180, and 153 are present in more than 85% of breast milk samples, indicating a wide distribution of these congeners as well as significant population exposure to them. Although all three were detected in most samples, the average concentration of PCB 52 (162 ng/g l.w.) was significantly higher than the average concentration of PCB 153 (3.52 ng/g l.w.) and PCB 180 (3.68 ng/g l.w.), which is similar to the pattern of frequency but not in concentrations of these congeners reported in studies from France, the Czech Republic and China (Brucker-Davis, 2010; Cajka, 2003; Cerná et al., 2010). These differences may be due to variations in the composition of the commercial PCB products used, including differences between lots of the same product, the analytical methods used in the studies, and ultimately the stability of the relative difference in metabolic kinetics of individual congeners in different environments. (Schulz, B., Carlson, L. M., Christensen, K., et al., 2024). High concentrations indicate the need for further identification and monitoring of active sources as well as the implementation of measures for the management of PCB-containing waste in accordance with the provisions of the Stockholm Convention (UNEP, 2012).

Lower concentrations of the multichlorinated congeners PCB 153 and PCB 180 indicate chronic exposure that can be explained by their higher stability, lower volatility and potentially increased consumption of fish as one of the exposure sources (Glynn et al., 2007).

The concentrations of PCB 180 and PCB 153 determined in this study are lower than those reported in studies conducted in Greece, Russia, Poland, Sweden, and France (Costopoulou et al., 2006; Polder et al., 2008; Jaraczewska et al., 2006.; Lignell et al., 2009, Brucker-Davis, 2010). Similar or lower levels of these congeners have been reported in Serbia, Turkey and Bulgaria (Vukavić et al., 2008; Erdogrul et al., 2004; Georgieva et al., 2023).

Comparing the results of our study with the data from the research conducted in Croatia, a slightly higher concentration of PCB28 was observed in our study. However, the concentrations of PCB52 and PCB101 were several times higher, while the values for PCB153 and PCB180 were slightly lower compared to the Croatian findings (Klinčić et al., 2014).

Concentrations of dioxin-like (dl-PCB) congeners (77, 123, 118, 105, 126, 167, 156, 157, 169) and non-dioxin-like (ndl-PCB) congeners (PCB30 and PCB 204) were also determined in the analysed milk samples. Among the ndl-PCBs, PCB30 was detected in more than 90% of the samples, while PCB204 was present in 13% of the samples. The dl-PCB congeners PCB118, PCB126 and PCB105 were present in 60% - 73% of samples, while congeners PCB77, PCB156, PCB157, PCB167 and PCB169 were present in 30% to 45% of the samples. Congener PCB123 was detected in only one sample and at a concentration below the detection limit, similar to what was reported in a study conducted in Italy (Aballe et al.,

2008), while concentrations of 0.23 ng/g l.w. were detected in Germany (Wittsiepe *et al.*, 2007)

A similar pattern in the frequency of the detected congeners was reported in the Croatian study (Klinčić *et al.* 2014). In our study, low average concentrations were recorded in the range from 1.00 to 7.32 ng/g l.w. for PCB congeners 105, 156, 157, 126, 204, 118, 77, while higher average concentrations were observed for PCB congeners 30 (21.16 ng/g l.w.), 169 (18.63 ng/g l.w.) and 167 (33.4 ng/g l.w.). The concentrations for PCB congeners 105, 156 and 118 in our study (0.54 ng/g l.w., 1.01 ng/g l.w. and 2.60 ng/g l.w., respectively) are similar to the concentrations of the same congeners in the Croatian study (0.9 ng/g l.w., 0.8 ng/g l.w. and 0.9 ng/g l.w.) with slightly higher concentrations of congener 118 in our study. In Italy (Abballe *et al.*, 2008) and Sweden (Lignell *et al.*, 2009), the concentrations of PCB 156 and PCB 157 were relatively higher compared to our and Croatian findings. A study conducted in China between 2003 and 2005 (Zhao *et al.*, 2007) showed concentrations of PCB 77 comparable to those in our study (5.15 ng/g l.w. vs. 5.72 ng/g l.w., respectively). However, significantly higher levels of PCBs 118 and 105 were detected in China compared to BiH and other European countries, which could be explained by intensive industrialization in certain parts of China.

In our study, the dl-PCB congeners PCB77 (5.72 ng/g fat), PCB126 (2.87 ng/g fat) and PCB169 (9.77 ng/g fat) were detected. These congeners were not detected or were detected in very low concentrations in various studies conducted across Europe between 1999 and 2011 (Abballe *et al.*, 2008; Lignell *et al.*, 2009; Bencko *et al.*, 2004; Wittsiepe *et al.*, 2007; Klinčić *et al.*, 2014). Variation in

detection can be attributed to different potential exposure sources, as well as to differences in the implementation of legal regulations on PCB use restrictions and waste management practices. Potential sources of exposure to dl-PCB congeners in BiH could be continued presence of old electrical equipment and transformers containing PCBs, industrial and post-war contamination of soil and air, frequent uncontrolled incineration of various types of waste (plastics, rubber), as well as insufficient compliance with regulations required under Stockholm and Basel Conventions, which BiH has formally committed to uphold.

Concentrations of PCB 169 were significantly higher ( $Z=-2.42$ ;  $p<0.016$ ) in mothers who were smokers (current or former) than in non-smokers. The sum of dl-PCB and ndl-PCB was significantly higher in the milk of mothers living in urban area comparing to those from rural areas. Significant negative correlation with mothers' parity was for PCB77 ( $p=0.028$ ), PCB118 ( $p=0.044$ ), and PCB156 ( $p=0.048$ ).

#### *Levels of selected OCPs in human milk samples*

In the analyzed breast milk samples, the average concentrations of the following OCPs were determined: alpha BHC, beta BHC, gamma BHC, heptachlor, aldrin, heptachlor epoxide isomer B, 2,4'DDD, dieldrin and 4,4' DDT (Table 4). When determining RF in spiked milk samples, RF of 4,4' DDT was low, but due to its toxicological relevance and presence in the population, it was included in further statistical processing.

Table 4 Descriptive statistics for selected OCPs (concentration in ng/g l.w.)

	N	Average	SE	Median	SD	Variance	Min	Max
<b>αBHC</b>	25	0.67	0.1	0.34	0.51	0.26	0.11	1.95
<b>βBHC</b>	26	6.98	3.96	1.8	20.22	408.73	0.6	104.22
<b>γBHC</b>	67	7.36	0.54	6.03	4.39	19.23	2.55	26.61
<b>HEPTACHLOR</b>	44	4.63	0.61	3.59	4.02	16.19	1.19	17.09
<b>ALDRIN</b>	6	0.27	0.07	0.16	0.17	0.03	0.16	0.5
<b>HEPTACHLOR EPOXIDE ISOMER B</b>	60	3.02	0.47	3.4	3.62	13.08	1.12	27.97
<b>2,4' DDD</b>	20	2.78	0.32	2.78	1.44	2.06	1.38	4.18
<b>DIELDRIN</b>	25	3.46	0.51	4.4	2.54	6.43	1.45	11.28
<b>4,4' DDT</b>	40	14.64	6.45	4.67	40.76	1661.54	1.54	251.62

OCP concentrations in breast milk samples from Bosnia and Herzegovina, shown in Table 4, compared to similar studies in Croatia and other European countries, indicate a moderate to elevated burden on the population. Compared to the findings in several Asian and African countries, OCP concentrations are significantly lower in our study. Regarding the concentrations of BHC isomers determined in our study, the levels of alpha BHC (0.34ng/g l.w) are similar to those reported in neighboring Croatia (0.40 ng/g

l.w.) (Klinčić *et al.*, 2014) and Poland (0.32 ng/g l.w) (Szyrwińska and Lulek, 2007). In contrast, beta BHC (1.80 ng/g l.w.) is almost twice lower than those in Croatia (3.10 ng/g l.w.), (Klinčić *et al.*, 2014) and several times lower than the levels in the Czech Republic (57 ng/g l.w.) (Cajka and Hajslova, 2003) and Poland (17.7 ng/g l.w.) (Szyrwińska and Lulek, 2007) and significantly lower than the extremely high values recorded in China (950 ng/g l.w.) (Wong *et al.*, 2002) and India (563 ng/g l.w.) (Bedi *et*

al., 2013). The detected concentration of lindane or gamma BHC (6.03 ng/g l.w) in this study is slightly higher compared to the results of the study conducted by Klinčić et al. (1.4 ng/g l.w.) and Szyrwińska et al. (0.16 ng/g l.w), similar to the values detected in Tunisia (8 ng/g l.w) (Ennaceur et. al., 2007) and several times lower than the extremely high values recorded in India (658 ng/g l.w) (Bedi et al., 2013). The concentration of 4.4' DDT of 4.67 ng/g l.w in our study is slightly higher than the level recorded in Croatia (1.5 ng/g l.w.), but several times lower than the levels recorded in China (545 ng/g l.w), Poland (70.30 ng/g l.w) and India (980 ng/g l.w) (Klinčić et al., 2014; Wong, et al., 2002; Szyrwińska and Lulek, 2007; Bedi et al., 2013). The average concentration of dieldrin detected in our study (3.46 ng/g l.w.) corresponds to the average concentrations of less than 3.8 ng/g l.w. reported by the Republic of Croatia (Fång et al., 2008) and Germany (Zietz et al., 2008). In breast milk samples from Latin American and Caribbean countries, the average concentrations of dieldrin ranged from 2.6 to 5 ng/g l.w., while in samples from African countries, the average values ranged from 1.3 to 4.1 ng/g of fat (UNEP 2009). A median concentration of 3 ng/g l.w. was reported in a study conducted in Japan and is also close to the values of dieldrin in our study. (Nagayama et al., 2007)

Heptachlor concentrations were, in most conducted studies, reported as cis-heptachlor epoxide (cis-HCL-epoxide), heptachlor epoxide (HCL-epoxide which is a mixture of A and B isomers) and heptachlor. The concentrations of heptachlor epoxide (HCL-epoxide) reported from European countries were in most cases below or close to 3 ng/g fat (UNEP 2011), which is similar to the levels of average concentrations of heptachlor and heptachlor epoxide (isomer B) recorded in our study (4.63 ng/g l.w. and 3.02 ng/g l.w. respectively). Higher concentrations of these analytes were recorded in Ukraine, 16–22 ng/g l.w. (Gladen et al., 1999 and 2003), and in the Netherlands, 30 ng/g l.w. (Albers et al., 1996). Significantly higher concentrations of beta BHC ( $p=0.045$ ), heptachlor epoxide ( $p=0.007$ ), and sum of OCP ( $p=0.002$ ) were found in the milk of mothers living in urban relative to mothers from rural areas.

#### Comparison of iPCB Sum Concentrations, Total PCB Sum and OCP Sum Between BiH Entities

A comparison of the average concentrations of the sum of selected analytes ( $\sum$ iPCB,  $\sum$ totalPCB and  $\sum$ OCP between the entities of BiH is shown in Chart 1.

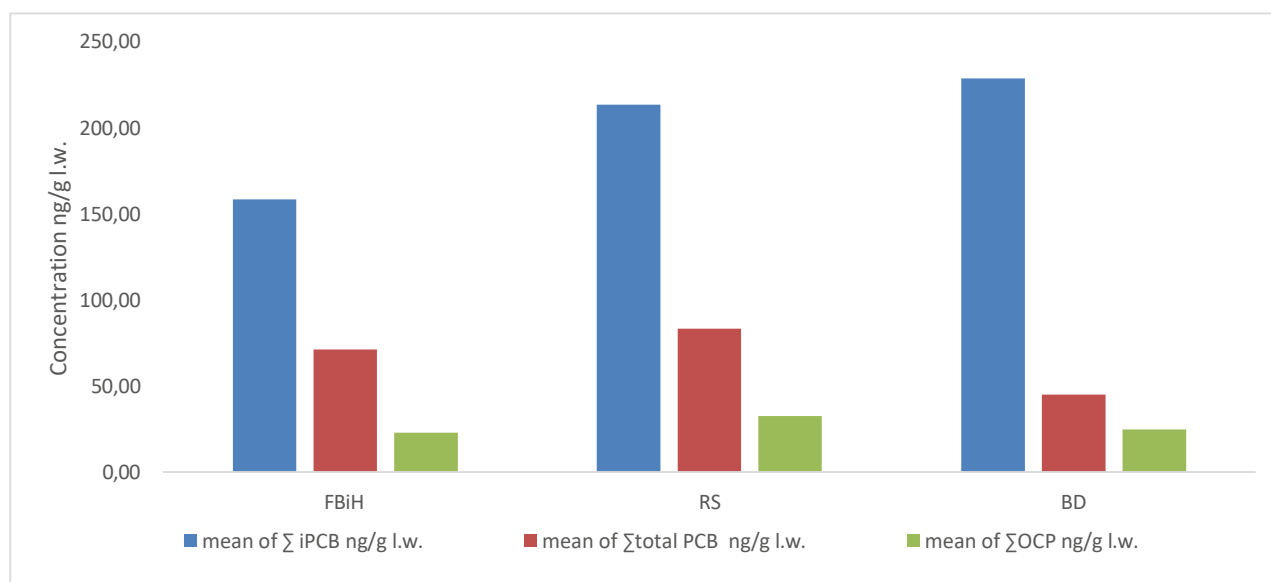


Chart 1 Average concentrations of the sum of selected analytes ( $\sum$ iPCB,  $\sum$ totalPCB and  $\sum$ OCP by entities (FBiH and RS) and district of BiH (Brčko District (BD))

Graph 1 shows clear differences in average concentrations of  $\sum$ iPCB, with the highest values present in Brčko District and Republika Srpska, while lower values of  $\sum$ iPCB are observed in the Federation of BiH. The Mann-Whitney U test showed that these differences between the two entities (FBiH and RS) were statistically significant ( $p=0.027$ ). Due to the limited number of samples ( $n=2$ ) from the Brčko District, data from this region were not considered in the statistical significance evaluation, despite the fact that the highest concentrations were recorded there. For the average concentrations of the  $\sum$ totalPCB and  $\sum$ OCP, the results of the Mann-Whitney U test showed that there was

no statistically significant difference between the entities ( $p>0.05$ ), although differences for the  $\sum$ totalPCB are visible on the graph.

#### CONCLUSION

Due to their potential adverse effects on human health, the monitoring of POPs—particularly through breast milk—is of great importance, especially in developing countries such as Bosnia and Herzegovina. The population is exposed to these contaminants through food, water, and air, and monitoring their levels in biological samples such

as breast milk provides valuable insight into both the degree of environmental contamination and the extent of exposure of mothers and newborns, who represent the most vulnerable population group.

Longterm monitoring of environmental pollutants also enables the assessment of temporal trends in environmental contamination and the evaluation of implemented protective measures and strategies. This further emphasizes the importance of continuous research on these substances.

According to available scientific literature, this study represents the first investigation of environmental pollutants through breast milk monitoring in Bosnia and Herzegovina. It is therefore of substantial importance for the country as a whole.

The measured concentrations of selected persistent pollutants in breast milk samples from Bosnia and Herzegovina fall within the range of values reported in the literature for urban areas and are lower than typical concentrations observed in contaminated regions. However, PCB congeners 52, 77, 126, and 169 were detected at higher levels compared to those reported in some of the European and Asian countries.

Significantly higher concentrations of beta BHC, heptachlor epoxide, sum of OCPs, sum of dl-PCB and ndl-PCBs were found in the milk of mothers from urban than from rural areas. Concentration of PCB169 was significantly higher in milk from mothers who were smokers (current or former) than in non-smoking mothers.

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**Summary/Sažetak**

Jedan od najvećih izazova današnjice je zagađenje okoliša, koje dovodi do ozbiljnih prijetnji ljudskom zdravlju i cjelokupnom ekosistemu. Zbog svoje visoke postojanosti i slabe biorazgradivosti, polihlorirani bifenili (PCB) i organohlorirani pesticidi (OCP), kao glavne kategorije okolišnih zagađivača antropogenog porijekla, ostaju prisutni u okolišu duži vremenski period, kontinuirano kružeći između zraka, vode i tla, te predstavljaju trajni rizik za javno zdravlje. Bosna i Hercegovina ranije nije provodila studije o prisutnosti okolišnih zagađivača u majčinom mlijeku, čiji bi rezultati mogli doprinijeti efikasnijoj zaštiti javnog zdravlja i poslužiti kao osnova za strateško planiranje usmjereno na smanjenje izloženosti ovim zagađivačima. Iz tog razloga, cilj ove studije bio je utvrditi nivoe odabranih postojanih organskih polutanata (PCB i OCP) u majčinom mlijeku dojilja (N=67) iz Bosne i Hercegovine (Federacija Bosne i Hercegovine, Republika Srpska i Brčko Distrikt). Ovdje prikazani rezultati prvi su te vrste na teritoriji naše zemlje. Prosječne vrijednosti kvantificiranih koncentracija PCB (17 kongenera) i OCP (8 spojeva) kretale su se u rasponu od 1.03 do 162.29 ng/g l.w., odnosno od 0.27 do 14.64 ng/g l.w. PCB kongeneri 52, 77, 126 i 169 su kvantificirani u višim koncentracijama u usporedbi s onima zabilježenim u nekim europskim i azijskim zemljama. U Brčko Distriktu i Republici Srpskoj pronađene su više vrijednosti u usporedbi s Federacijom Bosne i Hercegovine. Izmjerene koncentracije odabranih postojanih polutanata u uzorcima majčinog mlijeka prikupljenih u Bosni i Hercegovini uglavnom odgovaraju literaturnim podacima iz sličnih studija provedenih u zemljama regije i Evrope.