

## Levels of polychlorinated biphenyls in human milk samples in European countries

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Polychlorinated biphenyls (PCBs) are persistent pollutants, harmful to human health, which enter the human body mainly through food and bind to body fat. For these reasons their use in most countries is prohibited. Human milk has an advantage over other types of human samples in measuring human exposure to PCBs, as it is obtained with non-invasive sampling methods. In Europe, including Croatia, PCB levels have been monitored for many years. This review summarises PCB trends in human milk across Europe. The trend is generally downward, with higher levels prevailing in urban areas near industrial plants. The highest PCB levels were reported in the Czech Republic and Slovakia.

**KEY WORDS:** *breast milk, organochlorine compounds, PCBs, persistent pollutants*

Persistent organic pollutants (POPs) have been a serious threat to the environment and human health for a long time. POPs such as 1,1'-(2,2,2-trichloroethane-1,1-diyl)bis(4-chlorobenzene) (DDT) and polychlorinated biphenyls (PCBs) were prohibited in many industrialised countries as early as the 1960s and 1970s. Yet even today these compounds can be found in places as remote as Arctic, where they have never been utilised but have been transferred there owing to their physical-chemical properties, semi-volatility, and persistence (1, 2). Due to lipophilicity, they accumulate in the adipose tissue and accumulate along the food chain. This is why the highest levels are found in the top marine predators such as seals, whales, polar bears, sea birds, and humans (1).

To protect human health and the environment, the international community has responded to the POP threat with the Stockholm Convention on POPs, which was signed in 2001, became effective in May 2004, and has been ratified by more than 170 countries (2-4). Generally, the levels of polychlorinated biphenyls (PCBs) in human milk are higher in the more industrialised areas of Europe and Northern America than in Africa and Asia (4). In European countries the most abundant PCB, PCB-153, has been detected in the lowest levels in Belgium, Latvia, Italy, Norway, Poland and Sweden, while its highest levels were found in Czech Republic, and Slovak Republic (4).

PCBs are aromatic organic compounds, which consist of two benzene rings with 1 to 10 chlorine atoms (Figure 1) (6, 15). Their chemical formula is  $C_{12}H_{10-n}Cl_n$  (5, 15), where  $n$  is the number of chlorine atoms. Considering the

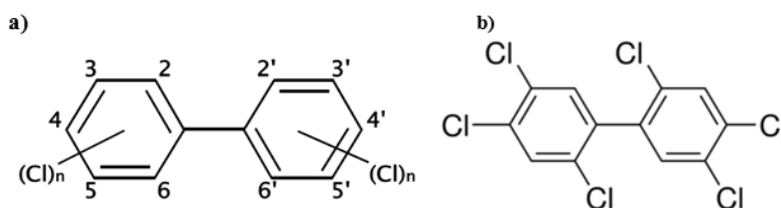
number and position of chlorine atoms, there are 209 isomers and PCB homologues, which are called congeners. All PCBs with the same number of chlorines are called homologues. A homologue with different substitution is called isomer. Table 1 lists the PCB homologues and the number of their isomers (5, 6). International Union of Pure and Applied Chemistry (IUPAC) has designated all PCBs with numbers from 1 to 209 (5-9).

In environmental samples, six indicator PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, and PCB-180) are most often determined due to their presence in commercial mixtures (Table 2). The toxicity of PCB congeners depends on the number and position of chlorine atoms in the molecule (7, 10). The most toxic congeners (Table 2) are non-*ortho* PCBs, which include PCB-77, PCB-81, PCB-126, and PCB-169. Other toxic PCBs are the mono-*ortho* PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, and PCB-189. PCB-60 and PCB-74 are also

**Table 1** Homologues of PCBs with corresponding number of isomers (5, 6)

PCB homologues (number of isomers)
Monochlorobiphenyl (3)
Dichlorobiphenyl (12)
Trichlorobiphenyl (24)
Tetrachlorobiphenyl (42)
Pentachlorobiphenyl (46)
Hexachlorobiphenyl (42)
Heptachlorobiphenyl (24)
Octachlorobiphenyl (12)
Nonachlorobiphenyl (3)
Decachlorobiphenyl (1)

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**Figure 1** Structure of: a) polychlorinated biphenyls and b) PCB-153 (2,2',4,4',5,5'-hexachlorobiphenyl) (6, 15)

very often analysed due to their presence in environmental samples (7).

To have greater uniformity in risk assessment and comparability of findings in different samples, the scientific community introduced toxic equivalency (TEQ). A TEQ of a particular PCB congener is calculated by multiplying PCB level with the Toxic Equivalent Factor (TEF) of that PCB. If TEQ is calculated for a mixture of PCBs, all individual TEQs are summed up. TEF expresses the toxicity of a PCB congener in relation to the most toxic form, dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), whose TEF is set at 1 (5, 10-13). Table 2 shows the TEFs of the most toxic PCBs congeners and 2,3,7,8-TCDD (12, 13).

The production and commercial use of PCBs began in the USA in 1929, and about at the same time in Europe (Germany and France) (7, 9). PCBs were applied for many years in various fields of industry as heat-exchanges fluid in electric transformer and capacitors, additives in paints, carbonless copy paper, and plastics production due to their chemical stability, low flammability, and high dielectric constant (7, 9, 10, 14). In the European Union, the production of PCBs is prohibited, and waste disposal is regulated by law. Russia ended their production in 1993 (7).

These compounds are very toxic to fish. While higher doses can cause death, lower PCB doses were reported to

interfere with spawning. PCBs also cause reproductive failure and suppression of the immune system in various wild animals. Humans are mainly exposed to PCBs through contaminated food (14). The adverse effects of PCB on human health have been addressed by a number of studies, which have shown that PCBs can act as endocrine disruptors, disturb homeostasis, and cause reproductive, immuno-, neuro-, and genotoxicity (6, 15–17).

Monitoring of organochlorine compounds in the Croatia started in 1975. At first, it was focused on analysing pesticides and later extended on determining total PCB levels (10). The most serious soil contamination with PCBs was recorded during the Croatian Homeland War in 1991 due to the damage of condenser batteries in the Zadar electric power substation (18).

PCBs released in the environment are very resistant to physicochemical and biological degradation and do not break down easily. They persist in the air, water, and soil and have a tendency to spread through the food chain and accumulate in all living organisms (2, 15, 17). In humans, PCBs mostly accumulate in the fat tissue, blood, and milk.

Human milk is a popular and reliable matrix for the determination of exposure levels in the human population (2) due to its availability and non-invasive sampling. PCB levels in human milk are usually expressed per amount of milk fat (lipid base; lipid weight), which allows comparison

**Table 2** Six indicator PCBs and the Toxic Equivalent Factors (TEF) for the most toxic PCBs and dioxin (6, 7, 12, 13)

Six indicator PCBs		PCB congeners	WHO <sup>1</sup> 2005 TEF
<b>non-ortho PCBs</b>			
		PCB-77 (3,3',4,4'-Tetrachlorobiphenyl)	0.0001
		PCB-81 (3,4,4',5-Tetrachlorobiphenyl)	0.0003
		PCB-126 (3,3',4,4',5-Pentachlorobiphenyl)	0.1
		PCB-169 (3,3',4,4',5,5'-Hexachlorobiphenyl)	0.03
<b>mono-ortho PCBs</b>			
PCB-28	(2,4,4'-Trichlorobiphenyl)	PCB-105 (2,3,3',4,4'-Pentachlorobiphenyl)	0.00003
PCB-52	(2,2',5,5'-Tetrachlorobiphenyl)	PCB-114 (2,3,4,4',5-Pentachlorobiphenyl)	0.00003
PCB-101	(2,2',4,5,5'-Pentachlorobiphenyl)	PCB-118 (2,3',4,4',5-Pentachlorobiphenyl)	0.00003
PCB-138	(2,2',3,4,4',5'-Hexachlorobiphenyl)	PCB-123 (2',3,4,4',5-Pentachlorobiphenyl)	0.00003
PCB-153	(2,2',4,4',5,5'-Hexachlorobiphenyl)	PCB-156 (2,3,3',4,4',5-Hexachlorobiphenyl)	0.00003
PCB-180	(2,2',3,4,4',5,5'-Heptachlorobiphenyl)	PCB-157 (2,3,3',4,4',5'-Hexachlorobiphenyl)	0.00003
		PCB-167 (2,3',4,4',5,5'-Hexachlorobiphenyl)	0.00003
		PCB-189 (2,3,3',4,4',5,5'-Heptachlorobiphenyl)	0.00003
		<b>Dioxin</b> (2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD))	1

<sup>1</sup>World Health Organization (WHO) 2005

with the levels measured in blood or fat tissue (7). Like any other method, however, determination of PCBs in human milk has its limitations. The samples can only be obtained from lactating women, which excludes other population groups. In addition, PCB levels in human milk depend on many factors. The most important are eating habits, as PCBs mostly enter the human organism through consumption of animal food. Then there is the mother's age, and lactation duration (7).

Thanks to the development of analytical methods, gas chromatography coupled with mass spectrometry in particular, their validation, and intra- and inter-laboratory quality controls, today it is possible to compare the measured levels of PCBs with a high degree of reliability (7, 10).

Over the last decades, human milk has been used as an important medium for measuring PCBs contamination in humans. Among the most important contaminants, there are six indicator PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180), which are the most abundant in commercial PCB mixtures such as AROCLOR (6).

In this paper we present an overview of the current knowledge about PCBs levels determined in human milk samples across Europe. We have also included some representative data from Russian studies, which were interesting for the comparison.

#### *Levels of PCBs in human milk in European countries*

The World Health Organization (WHO) has been running global surveys of polychlorinated dibenzo-p-dioxin (PCDD), polychlorinated dibenzofurans (PCDFs), and PCBs in human milk since 1987 (19). The latest among them, covering 52 countries, have shown that the highest levels of these compounds are associated with intense industrialisation. The highest levels of PCBs were recorded in Eastern and Western Europe. Europe reported high levels of the six indicator PCBs, and the highest were found in the Czech Republic and Slovakia. Although many Western and Eastern European countries also reported high levels of dioxin-like PCBs (dl-PCBs), the highest level was reported for Ukraine.

Globally, lower PCB levels were reported in the southern hemisphere. Elevated PCB levels in these countries were associated with closeness to industrial plants. The surveys also established a strong relationship between dl-PCBs and the six indicator PCBs (19). Table 3 shows the levels of the six indicator PCBs and the most toxic non-*ortho* PCBs in human milk in some European countries and the European part of Russia.

The monitoring of organochlorine compounds in human milk started in Sweden in 1967. According to Noren and Meironyte (20), they have shown a decreasing trend over 20 to 30 years. In the sixties they PCB levels increased, but their drop was visible in 1972. From then till 1997 total PCB levels dropped further by 30 %. Lignell et al. (21)

reported a further decline (from 3.9 to 8.6 % a year) in human milk samples (N=335) collected in the Uppsala County between 1996 and 2006. In another study (22), the same authors reported that the highest levels in the human milk samples collected from 2008 to 2010 were those of PCB-153, followed by PCB-138 and PCB-180. The highest levels of a non-*ortho* congener were those of PCB-126.

Glynn et al. (23) reported small differences in median organohalogen pollutant levels in human milk samples collected from 204 primiparas from four Swedish regions in 2000-2004. PCB-153, PCB-138, and PCB-180 had the highest levels. The lowest levels of mono- and di-*ortho* PCBs were determined in a rural area of the northern part of Sweden.

A statistically significant decline in dl-PCB levels in human milk samples from Sweden were also reported from 1972 to 2011. The decrease ranged from 5.8 to 7.6 % a year. Over the last ten years these levels decreased by 8.1-12 % (24).

Similar investigations were conducted in other countries in northern Europe (25, 26). In Denmark, human milk dl-PCB and the six indicator PCB levels (WHO-TEQ2005 dl-PCB=6.6 pg g<sup>-1</sup> milk fat, six ndl-PCB=162.8 ng g<sup>-1</sup> milk fat) were significantly higher than in Finland (WHO-TEQ2005 dl-PCB=4.6 pg g<sup>-1</sup> milk fat, sum of six ndl-PCB=104.0 ng g<sup>-1</sup> milk fat) (26). In France, median dl-PCB and the six ndl-PCBs (WHO-TEQ 2005 dl-PCB=4.3 pg g<sup>-1</sup> milk fat, sum of six ndl-PCB=85.2 ng g<sup>-1</sup> milk fat) were lower than in Danish and Finnish women (26).

Between 2002 and 2009, 612 human milk samples were collected in Norway (27). Forns et al. (27) analysed the association between 13 PCBs and behavioural problems in children during the first two years of life and found none.

Polder et al. (28) investigated PCBs levels in human milk samples collected from primiparas from northern (Tromsø) (N=10) and southern Norway (Oslo) (N=19) in 2000 and 2001. Tromsø is a town located in Northern Norway 400 km north of the Arctic Circle, while Oslo is 2000 km south. In Oslo two locations were included, one in the city centre and the other to the south-east of the city. The median of sum PCBs<sub>20</sub> (PCB-28, PCB-52, PCB-74, PCB-99, PCB-101, PCB-105, PCB-114, PCB-118, PCB-123, PCB-128, PCB-138, PCB-153, PCB-156, PCB-157, PCB-167, PCB-170, PCB-180, PCB-187, PCB-189 and PCB-94) was the highest in the Oslo centre. The long-term temporal trend (1970–2002) of PCBs in Norwegian human milk showed an increase in PCBs since the restrictions in 1972 until PCB ban in 1979. After that, PCB levels decreased 65 % between 1982 and 1991.

In two Norwegian and one Lithuanian region sixteen PCBs (PCB-28, PCB-52, PCB-77, PCB-101, PCB-105, PCB-114, PCB-118, PCB-126, PCB-138, PCB-153, PCB-156, PCB-157, PCB-169, PCB-170, PCB-180, PCB-189) were measured in human milk samples pooled from 10-12 women. Their results showed no significant differences

between the regions, which may be explained by similar dietary habits of their residents (29).

Between 1939 and 1990, Russia produced about 180,000 t of PCBs (30, 31) in two European cities: Novomoskovsk and Dzerzhinsk. These products were used as dielectrics in transformers and capacitors or paint additives and lubricants. Used PCB-containing oils were then burned in furnaces (such as the blast furnace of the Novolipetsk metallurgical combine). In the cities and their surroundings where PCBs were produced, high levels of these compounds were released into the environment and ended in the local food products. Women from Serpukhov, who worked in the plant or lived near it, had high levels of PCBs in their milk (30, 32). The Baikal region had two sources of PCB pollution: chlorine production industrial waste and (possibly) the transformer plant in Usolie-Sibirskoye. Human milk was monitored there in nine cities and one village from 1998 to 2004. The sum of twenty-eight PCB congeners ranged from 91 to 5,300 ng g<sup>-1</sup> milk fat. Women from Irkutsk (regional capital) stood out with very high levels of PCBs in their milk (30).

Bobovnikova et al. (32) reported that an important source of environmental pollution in Serpukhov (Russia) over 35 years was a plant which used PCBs for filling capacitors. Their Institute of Experimental Meteorology monitored PCBs concentrations in the air, snow cover, soil, water, and biological materials (vegetation and breast milk). Human milk of women who worked at the plant and living near to it had extremely high PCB levels ranging from 1,000 to 2,400 µg L<sup>-1</sup>. Women who lived 2 km from the plant but did not work in it had much lower PCB levels: 22–35 µg L<sup>-1</sup>.

Polder et al. (33) analysed twenty-three PCBs (PCB-28, PCB-52, PCB-66, PCB-74, PCB-99, PCB-101, PCB-105, PCB-110, PCB-114, PCB-118, PCB-128, PCB-138, PCB-141, PCB-149, PCB-153, PCB-156, PCB-157, PCB-170, PCB-180, PCB-187, PCB-194, PCB-206, and PCB-209) in thirty human milk samples collected in Murmansk and Monchegorsk (north-western part of Russia) in 1993. All samples were collected fourteen days after delivery. In Monchegorsk they found significantly higher levels of PCB-28, PCB-66, PCB-74, PCB-105, PCB-110, and PCB-118 than in Murmansk. In Murmansk they measured significantly higher levels of PCB-52, PCB-128, PCB-206, and PCB-209 than in Monchegorsk. No significant differences were discovered in the levels of non-*ortho* PCBs between the cities. PCB-74, PCB-99, PCB-118, PCB-138, PCB-153, and PCB-180 were the main congeners and accounted for 82 % of the sum of all PCB levels. The authors concluded that the significant difference in the PCB pattern between the two cities may suggest that the population of Monchegorsk was exposed to a mixture of PCBs with lower chlorine content.

Polder et al. (34) issued a very detailed report about 30 PCBs and other POPs in 140 human milk samples of primiparas and multiparas collected within three days after delivery at four sub-arctic and arctic locations in Russia

between 1996 to 1997. The most prevalent congeners were PCB-153, PCB-138, PCB-118, and PCB-180. Hexachlorinated PCBs accounted for more than 47 % of the sum of all measured PCBs. The levels of higher chlorinated PCBs and the PCB profile in human milk samples were explained with living conditions and dietary habits of the women who lived in these areas. The highest mean level of sum-PCBs (458 ng g<sup>-1</sup> milk fat) was determined in the multiparas of Narayan-Mar and in one primipara it was as high as 1090 ng g<sup>-1</sup> milk fat. POP levels varied significantly across the locations, which was also explained by differences in dietary habits between individuals and groups.

Polder et al. (31) also reported on spatial and temporal changes in PCB levels in human milk samples (N=42) in the north-western part of Russia characterised by heavy industry (the Kola peninsula and the area enclosing the White Sea) from 2000 and 2002 by comparing them with the corresponding levels determined between 1993 and 1996 (N=58). The median levels of ΣPCBs<sub>12</sub> (PCB-28, PCB-52, PCB-74, PCB-99, PCB-101, PCB-128, PCB-138, PCB-153, PCB-170, PCB-180, PCB-187 and PCB-194), Σmono-*ortho* PCBs<sub>4</sub> (PCB-105, PCB-118, PCB-156, and PCB-157), and ΣPCBs<sub>16</sub> (sum of PCB<sub>12</sub> and mono-*ortho* PCBs<sub>4</sub>) were the highest in Murmansk (median 316 ng g<sup>-1</sup> milk fat) and Kargopol (303 ng g<sup>-1</sup> milk fat) and the lowest in Arkhangelsk (175 ng g<sup>-1</sup> milk fat). Between 1993 and 2002, ΣPCBs<sub>12</sub>, Σmono-*ortho* PCBs<sub>4</sub>, and ΣPCBs<sub>16</sub> dropped 29, 34, and 30 % in Murmansk and 40, 53, and 43 % in Arkhangelsk, respectively. In Kargopol, however, the mean levels of ΣPCBs<sub>12</sub>, Σmono-*ortho* PCBs<sub>4</sub>, and ΣPCBs<sub>16</sub> increased 40, 16, and 36 %, respectively.

Bake et al. (35) were the first to assess levels of POPs in human milk in Latvia. They analysed 15 samples from the town of Olaine, with chemical industry, and compared their PCB levels with 15 control samples. The level of the six indicator PCBs in pooled samples of the Olaine group was higher than in control (141.8 ng g<sup>-1</sup> milk fat vs. 110.9 ng g<sup>-1</sup> milk fat). Even so, these are the lowest levels of the six indicator PCBs in Central Europe. The authors also observed that the level of sum PCBs correlated with body weight gain during pregnancy.

Gleden et al. (36) analysed PCB levels in human milk samples collected from 200 women from the cities of Kyiv (capital) and Dneprodzerzhinsk (a highly industrialised city) in Ukraine. The milk samples were analysed for two dl-PCBs (PCB-126 and PCB-169). The milk samples were pooled in four groups: by city and age (under 30 and 30 years and above). The results are shown in the Table 3. Individual samples from these women have been analysed for some PCB congeners in an earlier investigation (37).

Among other European regions, the Faroe Islands stand out with high POP and particularly high PCB levels in human milk (38). In fact, the PCBs levels were higher than in some European countries such as Sweden and Belgium. Samples were taken from 10 women, and total PCBs (PCB-



105, PCB-118, PCB-128/PCB-167, PCB-138, PCB-146, PCB-153, PCB-156, PCB-170, PCB-180, PCB-183, and PCB-187) varied from 2300 ng g<sup>-1</sup> milk fat in 1987 to 1600 ng g<sup>-1</sup> milk fat in 1997 and 1800 ng g<sup>-1</sup> milk fat in 1999, which could be explained by a high seafood intake, which includes pilot whale blubber, flumars, and flumar eggs contaminated with PCBs.

Čechova et al. (39) reported the median levels of the six indicator PCBs in 545 human milk samples from Slovakia (37 samples collected from 2010 to 2012), the Netherlands (120 samples collected between 2011 and 2014), and Norway (388 samples collected between 2001 and 2006). Slovakia reported the highest levels (144 ng g<sup>-1</sup> milk fat), followed by Norway (62.02 ng g<sup>-1</sup> milk fat) and the Netherlands (39.09 ng g<sup>-1</sup> milk fat).

In another study, Čechova et al. (40) analysed a total of 120 human milk samples collected between 2011 and 2015 in the Netherlands. The highest median level was obtained for PCB-153 (16.0 ng g<sup>-1</sup> milk fat), followed by PCB-180 and PCB-138 (10.7 ng g<sup>-1</sup> milk fat for both). In samples collected from 2009 to 2012 the levels and profiles of PCBs were comparable with other European countries such as Sweden, Belgium, or Croatia. Compared to the Czech Republic, however, these levels were about six to eight times lower.

Chovancova et al. (41) analysed the milk of primiparas (N=33) living near industrial areas in Slovakia, collected from 2006 to 2007 and determined twelve dl-PCB congeners. The highest levels were reported for the mono-ortho substituted PCB congeners PCB-118 and PCB-156, followed by PCB-167 and PCB-105. One of the reasons why the Slovakian PCB levels in milk were similar to those recorded in the Czech Republic could be that more than 11,600 t of PCBs were used as dielectric and heat exchanger fluids and paint additives in the former Czechoslovakia from 1959 to 1984 (41, 42).

The Czech Republic is one of the most PCB-polluted countries in the world. Long-term POP data suggest a trend toward decline in human milk in industrial and urban regions as well as in rural areas located near industrial zones (43). The authors also found the levels of monitored chlorinated compounds correlated with mother's age.

Černá and Bencko (44) also reported high PCB levels in human milk samples from the Czech Republic. The highest were found for PCB-153, while total PCBs ranged from 0.5 to 3.45 mg kg<sup>-1</sup> fat milk.

In a study of 90 human milk samples collected in seven urban areas of the Czech Republic from 1999 to 2000, Černá et al. (45) found the six indicator PCBs in all analysed samples. Uherske Hradiště stood out as a risk area because of its closeness to an old industrial plant which produced PCB paints in the 1970s and 1980s (45, 46).

Long-term trends of POPs, in human milk, PCBs in particular, continued to be monitored in the Czech Republic after the prohibition, between 1996 and 2011. Banyiova et al. (47) calculated daily PCB intakes from the levels of

PCBs in human milk reported by the Czech Human Biomonitoring (CZ-HBM) study (48) and compared them with the food-derived daily intakes for PCBs reported by the National Institute of Public Health (49). The calculated daily intake was in good agreement with the reported dietary PCB intake data, which confirms food as the main source of PCB exposure. Yet, even though the long-term trends in daily PCB intake were decreasing since the ban, some years showed increased PCB levels in human milk, which points to a more recent food contamination. The authors therefore stressed the need for continuous monitoring of POP levels in the Czech Republic.

In Poland, Jaraczewska et al. (50) analysed human milk samples collected from 22 women in the second half of the 2004. In spite of the limited sample size, it was safe to assume that the PCB levels were at the low end of the European range.

Szyrwinska et al. (51) analysed the levels of seven PCBs (the six indicator PCBs and PCB-118) in 27 human milk samples collected from Polish women living in the Wielkopolska province from 2000 to 2001 (51). They also reported relatively low mean and median levels of the sumPCBs<sub>7</sub> (114.8 and 90.3 ng g<sup>-1</sup> milk fat in primiparas, and 101.8 and 71.7 ng g<sup>-1</sup> milk fat in secundiparas) in comparison with the Czech Republic, Slovakia, and Spain.

Hernik et al. (52) analysed the levels of PCB-77, PCB-101, PCB-118, PCB-126, PCB-138, PCB-153, PCB-170, and PCB-180 in 28 human milk samples collected in the Warsaw region. The highest levels were obtained for PCB-153 (39.8 ng g<sup>-1</sup> milk fat), PCB-180 (26.1 ng g<sup>-1</sup> milk fat), and PCB-138 (23.6 ng g<sup>-1</sup> milk fat). These were comparable with the levels reported for Poland by other authors and with other European countries.

Vigh et al. (53) analysed PCBs levels in samples of human milk (N=22) collected in the Baranya Country in Hungary on days 5, 12, and 84 postpartum (53). They detected twelve dl-PCBs and seven ndl-PCBs and established an inverse correlation between the duration of lactation and the levels of several PCB congeners. The most notable decline was observed between postpartum days 5 and 12. On postpartum day 5, total ndl-PCB level (the six indicator PCBs and PCB-170) was 33.5±29.2 ng g<sup>-1</sup> milk fat. By day 12 it dropped to 27.4±20.6 ng g<sup>-1</sup> milk fat (18 % decrease) and by day 84 to 26.9±24.8 ng g<sup>-1</sup> milk fat (20 % decrease). A similar decreasing trend was observed for dl-PCBs and total PCB levels.

In Germany, Furst et al. (54) analysed more than 1,400 human milk samples from 1984 to 1991 in Germany. PCBs levels had been constant for a long time, while a slight decrease was observed from 1989 to 1991, possibly due to the ban.

Zietz et al. (55) reported the results regarding a long-term biomonitoring of PCBs levels in 4314 human milk samples collected in northern Germany from 1999-2006. Median levels of total PCBs showed a clear rise with the

increasing age of mothers, but the overall trend was showing decline.

Still in Germany, the Bavarian Health and Food Safety Authority has been monitoring organohalogen compounds in human milk samples for more than 20 years (56). Data on indicator PCBs are accessible since 1985. Monitoring of sum PCBs (PCB-138, PCB-153, and PCB-180) showed a decreasing trend over the 1985-2005 period.

Raab et al. (57) also reported on organochlorine compounds levels in 516 human milk samples collected in seven Bavarian regions from 2007 to 2008. Median levels of the sum six indicator PCBs was 150 ng g<sup>-1</sup> milk fat, while the median of the WHO-TEQ1998 dl-PCBs was 6.4 pg g<sup>-1</sup> milk fat.

Wittsiepe et al. (58) in their "Duisburg birth cohort study" analysed blood and human milk samples collected from 169 participants who lived in an industrialised area of Germany between September 2000 and January 2003. They reported a good correlation between PCBs levels, expressed on lipid base, in milk and blood. The levels of WHO-TEQ (PCB) were in the range from 1.40 to 42.23 pg g<sup>-1</sup> lipid base for blood (median: 10.81, arithmetic mean: 11.57) and from 1.21 to 50.10 pg g<sup>-1</sup> milk fat for milk (median: 13.00, arithmetic mean: 13.43). The distribution between blood and milk depended on the molecular weight of the substances. The levels of higher chlorinated PCBs were two to four times higher in blood than in milk, while the levels of lower chlorinated PCBs were two times higher in milk than in blood.

Brucker-Davis et al. (59) found a significant positive correlation between PCB-153 and  $\sum$ PCB levels in umbilical cord blood (N=84) and milk (N=69) samples collected in a French maternity hospital in 2002-2005. The median PCB-153 content in cord blood was 0.2 ng mL<sup>-1</sup> (range: 0.1-2.6 ng mL<sup>-1</sup>) and in milk 59.0 ng g<sup>-1</sup> of milk fat (0.5-1395.3 ng g<sup>-1</sup>).

In a pilot study carried out in France before the start of the Elf project (French longitudinal study from childhood) Focant et al. (60) collected 44 human milk samples six to eight weeks after delivery and measured twelve dl-PCBs (eight mono-*ortho* PCBs and four non-*ortho*-PCBs), and the six indicator ndl-PCBs. The levels of dl-PCBs were at the high end of the European range and so were the ndl-PCBs but this was still significantly lower than ndl-PCB levels reported for the Slovak and Czech Republic.

Croes et al. (61) analysed PCBs in 84 human milk samples collected in the rural areas in Flanders (Belgium) in 2009-2010. The analysis included PCB-118, PCB-138, PCB-153, PCB-170, and PCB-180, and dl-PCBs in individual samples and the pooled sample. The levels of PCB-31, PCB-52, PCB-95, and PCB-149 were below the limits of quantification (LOQ) in all individual samples. The levels of other PCBs were lower than in the WHO Human Milk Study from 2006 (61).

In the United Kingdom, Kalanatzis et al. (62) analysed 54 human milk samples collected between late 2001 and

early 2003. The 27 samples collected in London showed consistently higher PCB levels than the 27 samples collected in Lancaster.

In Ireland, Pratt et al. (63) reported a decreasing trend in dl-PCB levels in human milk samples collected from 2002 to 2010. The total levels of the six indicator PCBs in 11 pooled samples ranged between 31.5 and 59.0 ng g<sup>-1</sup> milk fat in 2010 and between 37.4 and 64.5 ng g<sup>-1</sup> milk fat in 2002. The 2010 total dl-PCB levels were also generally lower than those measured in 2002. The measured levels were also lower than those found in other European countries, perhaps because Ireland had not had a history of extensive industrial development.

In Italy, a large number of studies have been carried out to determine PCBs in human milk. Ingelido et al. (64) analysed pooled milk samples collected in Venice (April 1998 – October 2000) and Rome (January 2000 – July 2001). They found ndl-PCBs (PCB-28, PCB-52, PCB-101, PCB-122, PCB-124, PCB-128, PCB-138, PCB-141, PCB-153, PCB-170, PCB-180, PCB-183, PCB-187, PCB-194, PCB-206, and PCB-209). The criteria for selecting congeners for analysis were relative profusion in human tissues, toxicological relevance, and distribution in the environment. Mothers from Venice were divided into three groups according to their consumption of marine food to see if there was an association between marine food and PCB exposure. The most abundant was PCB-153, followed by PCB-138 and PCB-180. There was no association between marine food consumption and PCB levels in milk.

Abballe et al. (65) also analysed organochlorine compounds in pooled human milk samples of Venetian and Roman women in 1998–2001 period. They found a decrease in dl-PCBs 77 and 126 of around 52 % compared to the 1987 measurements reported by Schechter et al. (66) and Larsen et al. (67), while dl-PCB-169 showed a small decrease (26 %). Mono-*ortho* dl-PCBs decreased about 39 % but the dl-PCB-114 decreased the most (62 %). These trends are in agreement with reports from other European countries.

Ulaszewska et al. (68) analysed the levels of 19 PCBs in milk samples collected from in the residents of Giugliano, Milan, and Piacenza from April 2008 to November 2009. The most abundant congeners among dl-PCBs were PCB-170, PCB-118, and PCB-156, while PCB-153, PCB-180, and PCB-138 were the most abundant among the indicator PCBs. The most represented classes on all three locations were hexa- and heptachlorines.

The areas of Caserta and Naples in the Italian region of Campania had long suffered heavy environmental contamination with dl-PCBs due to a large number illegal waste dumps and the practice of burning waste. This is why Rizzi et al. (69) reported higher levels of dioxins and dl-PCBs in human milk samples from Caserta and Naples compared to those reported for Giugliano, Milan, and Piacenza (68).

Alivernini et al. (70) determined 37 PCB congeners in human milk samples collected in Rome in 2005-2007 and observed a decreasing trend in view of the previous two studies conducted in 1987 and 2000-2001.

In 2007, Schuhmacher et al. (71) measured PCB levels in the milk collected from women living near a waste incinerator in Catalonia, Spain. The levels of the most toxic congeners, PCB-126 and PCB-169, were 34.8 pg g<sup>-1</sup> and 26.1 pg g<sup>-1</sup> milk fat, respectively. The highest levels were measured for PCB-53 and PCB-180. The observed decreases in PCB levels corresponded to the reduction in the estimated dietary intake of these pollutants.

In a study with the same population in 2012, Schuhmacher et al. (72) analysed 20 milk samples, whose total PCBs ranged from 26.9 to 405 pg g<sup>-1</sup> milk fat (WHO-TEQ 0.71-5.28 pg g<sup>-1</sup> milk fat). The decrease in both planar and total PCBs in milk continued. They also reported that milk PCBs in women who lived in urban areas were 26 % higher than in women from the industrial areas (26 %).

In another Spanish study, Gomara et al. (73) measured the levels of 24 PCBs in human milk samples collected from nine women in 2005 and found that the PCB levels were similar to other reports since 2000 but lower than those before 2000. They were also in the range reported for other European countries.

In Croatia, the levels of PCBs in human milk have been monitored for over thirty years. Krauthacker et al. (74) reported that PCBs in samples collected from healthy mothers in urban or semi urban locations in Croatia (Jastrebarsko, Osijek, and Zagreb) decreased about 50 % between 1981 and 1989, while the decrease slowed down between 1990 and 2000. In human milk samples from the island of Krk and Croatian capital Zagreb collected in 2000 PCB levels were similar. The highest levels were reported for PCB-153, PCB-138, and PCB-180 (75).

Klinčić et al. (76) analysed the levels of 20 PCBs in human milk samples collected in Zagreb and the coastal town of Zadar from 2009 to 2011. A comparison with results obtained ten years earlier (75) showed a decreasing trend in the samples collected in Zagreb. Mothers from Zadar had higher TEQ values for mono-*ortho* PCB congeners. The authors associated greater PCB exposure of women in the Zadar area with the contamination outbreak in the 1990s. The Croatian TEQ values were several times lower than in the Czech Republic and Slovakia. The authors also concluded that infants consuming mothers' milk were not at risk of adverse effects of the PCBs.

In another Croatian study (77), 20 PCBs were determined in human milk samples collected from 33 multiparas from Zadar in 2011. The most common congeners were PCB-153, PCB-138, PCB-180, and PCB-170. PCB-126 was the most common non-*ortho* PCB. PCB-153 was the most abundant congener in the milk samples, followed by PCB-180, PCB-138, and PCB-170. These findings placed Croatia at the middle of the PCB ranges in human milk reported for other countries. As the

daily intakes of all the determined compounds through milk were below tolerable limit, they posed no risk for mothers and breastfed infants.

So far, Slovenia has not produced a report on PCBs in human milk, but has set up human biomonitoring (78).

In Greece, Costopolou et al. (79) collected human milk and blood serum samples in urban (Athens) and rural (Kozani) areas from 2002 to 2004. PCB levels in blood serum samples were significantly higher in the samples from Athens than from Kozani. Blood and milk levels showed a similar pattern. Overall, however, mean PCBs were lower than those measured in other European countries.

## CONCLUSION

Due to their harmful effects on human health, PCB levels in human milk have been monitored in Europe for a long time and a lot of data have been collected during that time, especially since the Stockholm Convention.

Most of the studies focused on the six indicator PCBs because of their large contribution to the AROCLOR commercial mixtures. The most dominant are PCB-138, PCB-153 and PCB-180, while PCB-153 is the most abundant in human milk samples. The most toxic non-*ortho* PCBs (PCB-77, PCB-81, PCB-126 and PCB-169) have also been regularly monitored in human milk samples. The highest European levels were reported in the Czech Republic and Slovakia, most likely due to heavy industrial production. Croatia is placed somewhere in the mid-range. Thanks to the ban, PCB levels in all European countries are slowly decreasing.

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## Conflicts of interest

None to declare.





Table 3 continued

France (five different countries)	2007 (44)	Mean	7.78	15.75	8.29	39.97	83.04	48.34	$\sum_6$ PCBs: 203.18	PCB-77: <LOQ PCB-81: 10 PCB-126: 70 PCB-189: 30	60
Germany (Duisburg)	2000-2003 (169)	Median	ND	ND	ND	51	81	42	ND	(PCB-126: 67.3; PCB-169: 28.9)	58
Greece (Athens)	2003 (8)	Mean	1.12	0.73	0.86	24.00	43.90	23.80	$\sum_6$ PCBs: 94.4	(PCB-77: 0.00; PCB-81: 0.00; PCB-126: 2.84; PCB-169: 0.24)	79
Hungary (Baranya County)	2007 (22)	Mean (day 5 postpartum)	1.38	1.38	2.82	8.79	12.76	4.27	$\sum_6$ PCBs+ PCB-170): 33.52	(PCB-77: 37.4; PCB-81: 2.1; PCB-126: 5.9; PCB-169: 11.0)	53
Ireland	2010 (109)	Conc. of overall pool	0.77	0.08	0.26	6.9	12.6	7.4	ND	(PCB-77: 1.62; PCB-81: 0.9; PCB-126: 17.5; PCB-169: 9.21)	63
Italy (Piacenza; Milan; Giugliano)	2008-2009 (22; 16; 21)	Mean (Piacenza; Milan; Giugliano)	1.20 1.58 0.22	0.27 0.28 0.15	0.36 0.45 0.25	20.81 10.70 15.86	37.82 21.85 33.10	31.36 14.77 15.57	$\sum_6$ PCBs: 113.69; 65.47; 72.42) $\sum_9$ PCBs: 130.98; 82.18; 94.15)	PCB-77:30, 70.3; PCB- 81:20,80.3; PCB-126:30, 30.40; PCB-169: 60; 80; 20	66
Italy (Roma)	2000-2001 (10)	Conc. in human milk sample pools (Roma)	3.5	0.26	0.59	58	77	56	$\sum_{16}$ PCBs: 240	ND	64
Italy (Roma)	2005-2007 (30)	Mean	0.80	0.15	0.40	32.5	53.5	32.1	$\sum_6$ PCBs: 119	ND	68



Table 3 continued

	Median (Tromsø)	2.3	0.4	0.8	35	52	22	$\Sigma_6$ PCBs: 112	ND
	Mean (Tromsø)	2.4	0.5	0.9	36	53	23	$\Sigma_6$ PCBs: 115	ND
	Median (Oslo GRÜ)	2.0	0.2	0.8	40	54	26	$\Sigma_6$ PCBs: 125	28
	Mean (Oslo GRÜ)	2.4	0.3	0.7	39	52	26	$\Sigma_6$ PCBs: 119	ND
	Median (Oslo SNO)	3.4	0.2	0.7	36	46	21	$\Sigma_6$ PCBs: 108	ND
	Mean (Oslo SNO)	3.9	0.2	0.8	34	44	20	$\Sigma_6$ PCBs: 102	ND
	Levels of pooled milk samples (Tromsø)	7.4	4.0	0.6	88.7	127.5	44.4	$\Sigma_6$ PCBs: 273	(PCB-77: 8.5; PCB-126: 150.5; PCB-169: 107.1)
	Levels of pooled milk samples Hamar	7.6	<0.7	<0.4	86.1	127.9	42.9	$\Sigma_6$ PCBs: 265	(PCB-77: 5.2; PCB-126: 70.2; PCB-169: 38.9)
	Levels of pooled milk samples (Skien/Porsgrunn)	6.7	2.9	1.1	94.4	140.8	55.7	$\Sigma_6$ PCBs: 302	(PCB-77: 5.7; PCB-126: 61.7; PCB-169: 48.9)
	Primiparae Median	0.88	1.13	0	27.0	36.3	16.6	$\Sigma_7$ PCBs: 90.3	
	Primiparae Mean	0.81	1.11	0.03	35.3	45.6	24.4	$\Sigma_7$ PCBs: 114.8	ND
	Secundiparae Median	0.92	1.06	0	20.7	29.5	16.9	$\Sigma_7$ PCBs: 71.7	51
	Secundiparae Mean	0.92	1.1	0.17	28.8	42.1	21.6	$\Sigma_7$ PCBs: 101.8	

Table 3 continued

Russia (Murmausk; Monchegorsk)	Median (Murmansk)	2.8	3.8	3.2	85.1	115.3	33.7	$\sum_{23}$ PCBs: 429	pooled sample (PCB-77: 160.4; PCB-126: 113.1; PCB-169: 29.4)	33
	Mean (Murmansk)	4.1	4.5	3.7	97.1	127.0	37.9	$\sum_{23}$ PCBs: 429.4		
	Median (Monchegorsk)	4.4	1.3	3.4	88.5	112.0	30.1	$\sum_{23}$ PCBs:511	pooled sample (PCB-77: 121; PCB-126: 137.6; PCB-169: 40.9)	
	Mean (Monchegorsk)	6.0	1.9	4.4	95.6	118.0	31.1	$\sum_{23}$ PCBs: 490.5		
Russia (Murmansk; Arkhangelsk; Kargopol)	Median (Murmansk)	3	1	2	69	81	22	$\sum_{12}$ PCBs: 262		
	Mean (Murmansk)	5	2	3	80	89	26	$\sum_{12}$ PCBs: 281		
	Median (Arkhangelsk)	3	0.8	1	39	46	13	$\sum_{12}$ PCBs: 144		31
	Mean (Arkhangelsk)	3	1	1	43	53	16	$\sum_{12}$ PCBs: 157		ND
	Median (Kargopol)	3	1	6	61	67	28	$\sum_{12}$ PCBs: 251		
	Mean (Kargopol)	22	1	11	57	70	42	$\sum_{12}$ PCBs: 302		
Slovakia (Krompachy)	Median	ND	ND	ND	ND	ND	ND	ND	(PCB-77: 3.7; PCB-81: 2.9; PCB-126: 32.5; PCB-169: 21.6)	41
	Mean	ND	ND	ND	ND	ND	ND	ND	(PCB-77: 4.7; PCB-81: 3.1; PCB-126: 53; PCB-169: 27.3)	
Slovakia	Median	1.11	0.13	0.22	30.70	59.09	44.32	$\sum_{6}$ PCBs: 144.10		39
	Mean	1.38	0.15	0.28	40.70	68.62	54.45	$\sum_{6}$ PCBs: 165.57		ND



Table 3 continued

Spain (Madrid)	2005 (9)	Median	0.51	1.6	1.1	12	33	20	$\sum_{24}$ PCBs: 103	(PCB-77: 320; PCB-81: 170; PCB-126: 34; PCB-169: 58)	73	
		Mean	0.89	1.6	1.8	16	39	22	$\sum_{24}$ PCBs: 105	(PCB-77: 320; PCB-81: 170; PCB-126: 52; PCB-169: 96)		
Spain (Catalonia)	2012 (20)	Mean	ND	ND	ND	ND	121	95	$\sum_{37}$ PCBs: 156	(PCB-126: 16.3; PCB- 169: 17.3) $\sum_{\substack{47,81,126,169}} \\ \text{PCBs: 42.9}}$	72	
Sweden (Stockholm region)	1972-1997 (135; 40)	Concentration of pooled samples 1972	34	4	11	190	215	88	$\sum_{14}$ PCBs: 1090		ND	20
		Concentration of pooled samples 1997	4	0	1	57	73	37	$\sum_{14}$ PCBs: 324			
Sweden (Stockholm region)	1972-2011 (75; 11)	Concentration of pooled samples 1972	ND	ND	ND	ND	ND	ND	ND	(PCB-77: 150; PCB-81: 17; PCB-126: 240; PCB-169: 92)		24
		Concentration of pooled samples 2011	ND	ND	ND	ND	ND	ND	ND	(PCB-77: <14; PCB-81: <1.8; PCB-126: 17; PCB-169: <9.2)		
Sweden (Uppsala County)	1996-2006 (335)	Median	1.8	most samples <LOQ	most samples <LOQ	26	52	25	ND	PCB-126: 39; PCB-169: 20		21
		Mean±SD	2.8±3.9	most samples <LOQ	most samples <LOQ	29±13	58±28	28±13	ND	PCB-126: 44±22; PCB-169: 22±12		



Table 3 continued

United Kingdom (London; Lancaster)	2001-2003 (27; 27)	Median	2.0	ND	ND	37	49	25	$\sum_{15}$ PCBs: 180	ND	62
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**non-ortho PCBs** (the most toxic congeners): PCB-77, PCB-81, PCB-126, PCB-169,  $\sum_3$ PCBs: The results of PCB congener CB-52 level are excluded from the total data of the group to avoid possible higher results because of the overlapping of chromatography peaks (35).  $\sum_6$ PCBs: six indicator PCBs: PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, and PCB-180.  $\sum_6$ PCBs: six indicator PCBs and PCB-118 (51, 59).  $\sum_{17}$ PCBs: (PCB-105, PCB-118, PCB-128/PCB-167, PCB-138, PCB-146, PCB-153, PCB-156, PCB-170, PCB-180, PCB-183, and PCB-187) (38).  $\sum_{17}$ PCBs: (PCB-28, PCB-52, PCB-74, PCB-99, PCB-101, PCB-128, PCB-138, PCB-153, PCB-170, PCB-180, PCB-187, and PCB-194) (31).  $\sum_{14}$ PCBs: PCB-28, PCB-52, PCB-101, PCB-105, PCB-114, PCB-118, PCB-138, PCB-153, PCB-156, PCB-167, PCB-170, PCB-180, PCB-183, PCB-187, PCB-194 and PCB-203) (62).  $\sum_{16}$ PCBs: PCB-28, PCB-52, PCB-101, PCB-122, PCB-124, PCB-128, PCB-138, PCB-141, PCB-153, PCB-170, PCB-180, PCB-183, PCB-187, PCB-194, PCB-206 and PCB-209) (64).  $\sum_{19}$ PCBs: all-PCBs + six PCBs indicator congeners: PCB-28, PCB-52, PCB-81, PCB-77, PCB-101, PCB-123, PCB-118, PCB-114, PCB-105, PCB-126, PCB-153, PCB-138, PCB-167, PCB-156, PCB-157, PCB-180, PCB-170, and PCB-189) (66).  $\sum_{23}$ PCBs: PCB-28, PCB-52, PCB-66, PCB-74, PCB-99, PCB-101, PCB-105, PCB-110, PCB-114, PCB-118, PCB-128, PCB-138, PCB-141, PCB-149, PCB-153, PCB-156, PCB-157, PCB-170, PCB-180, PCB-187, PCB-194, PCB-206, and PCB-209) (33).  $\sum_{29}$ PCBs: PCB-28, PCB-52, PCB-77, PCB-81, PCB-95, PCB-101, PCB-105, PCB-114, PCB-118, PCB-123, PCB-126, PCB-132, PCB-138, PCB-149, PCB-153, PCB-156, PCB-157, PCB-167, PCB-169, PCB-170, PCB-180, PCB-183, PCB-189, and PCB-194) (73).  $\sum_{37}$ PCBs: PCB-18, PCB-18, PCB-28/31, PCB-33, PCB-47, PCB-49, PCB-51, PCB-52, PCB-60, PCB-66, PCB-74, PCB-77, PCB-81, PCB-99, PCB-101, PCB-105, PCB-110, PCB-114, PCB-118, PCB-122, PCB-123, PCB-126, PCB-128, PCB-138, PCB-141, PCB-153, PCB-156, PCB-157, PCB-167, PCB-169, PCB-170, PCB-180, PCB-183, PCB-187, PCB-189, PCB-194, PCB-206, and PCB-209) (72). **di-ortho PCBs**: sum of PCB-153, PCB-138, and PCB-180) (76). ND - not determined and no data; LOD - limit of determination; LOQ - limit of quantification

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### Razine polikloriranih bifenila u uzorcima ljudskog mlijeka u europskim zemljama

Poliklorirani bifenili (PCB) dobro su poznati, dugo primjenjivani spojevi koji su štetni za ljudsko zdravlje. Ljudi su im uglavnom izloženi putem hrane budući da su to spojevi koji se dugo zadržavaju u okolišu. Kad dospiju u ljudski organizam zbog lipofilnog svojstva, vežu se za masti. Danas je njihova upotreba u mnogim zemljama zabranjena. Određivanje tih spojeva u uzorcima ljudskog mlijeka ima velike prednosti s obzirom na to da je uzorkovanje mlijeka neinvazivna tehnika, a samo mlijeko, zbog lipofilnog svojstva, medij pogodan za određivanje izloženosti čovjeka PCB-ima. Najveće razine PCB-a u ljudskome mlijeku izmjerene su u urbanim područjima koja su bila u blizini industrijskih pogona. Od europskih zemalja, najveće razine PCB-a u ljudskom mlijeku pronađene su u Češkoj i Slovačkoj. U ovom radu prikazani su podaci o raspodjeli i razinama PCB-a u uzorcima majčina mlijeka u europskim zemljama u kojima se već godinama kontinuirano smanjuje njihova koncentracija.

KLJUČNE RIJEČI: *majčino mlijeko; organoklorovi spojevi; PCB-i; postojani organski onečišćivači*