

Polychlorinated biphenyls in cow's milk, feed and soil in selected areas of Slovakia

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Article Details: Received: 2020-09-07 | Accepted: 2020-10-19 | Available online: 2020-12-31

<https://doi.org/10.15414/afz.2020.23.04.241-247>



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The contents of polychlorinated biphenyls (PCBs) were determined in raw cow's milk, feed and soil samples from three regions of Slovak Republic (SR) depending on the environmental regional classification of SR. Total 60 pool milk samples from undisturbed environment (Novoť area), moderately disturbed environment (Tulčík area), and from strongly disturbed environment (Čečejoyce area) collected in April, July and September were extracted, purified by gel permeation chromatography (GPC) and analyzed using the gas chromatography with electron capture detector method (GC-ECD). Thirty feed samples of total mixed ration (TMR) were collected in April and September and after the extraction and purification by GPC were analyzed using gas and liquid chromatography (GLC). Fifteen soil samples collected in April were analyzed using the GC-ECD. All samples of raw milk, feed and soil were under the limit of quantification (LOQ) from all regions and did not exceed the limits set by European Commission. There were no seasonal differences in the PCBs levels in the milk, feed and soil samples. The milk PCBs consumption from these locations has no negative impact on consumer's health. The results indicate the decreasing trend in PCBs occurrence in the environment and food sources. Despite this positive findings, there is a constant need to monitor environmental burden of PCBs in the different regions of Slovakia where mainly food of animal origin is produced and to recognize another sources of PCBs that may impact the food chain.

Keywords: polychlorinated biphenyls, PCB, cow milk, cows, Slovakia

1 Introduction

The persistent organic pollutants (POPs) are known to have a high potential of bioaccumulation in the animal and human organisms. Polychlorinated biphenyls (PCBs) are one of the POPs group highly resistant to degradation. These lipophilic compounds accumulate preferentially in the adipose tissue of animals (Hirako, 2008), human white adipose tissue and lipid droplets in fat cells (Müllerová and Kopecký, 2007; Bourez et al., 2012). Due to their toxic potential, the public and health concerns are still in the focus of the current research. The PCBs are confirmed carcinogens for humans. The malignant melanoma, non-Hodgkin lymphoma and breast cancer were observed (IARC, 2016). However, a variety of adverse effects of the so called "dioxin-like" PCBs have been observed. They cause the reproductive disorders in men and women (Toft, 2014; Sumner et al., 2019), liver dysfunctions (Boucher et al., 2015; Deng et al., 2019),

immunosuppression (Leijds et al., 2009), neurological effects (Giuda et al., 2017; Berghuis and Rose, 2019) and thyroid disorders (Hagmar et al., 2001; Gaum et al., 2019).

Food is the main source of human exposure to PCB (>90%) with foods of animal origin usually being the predominant sources (Cederberg, 2000). Feed is the major source of PCBs intake by farm, food producing animals and PCBs are transferred into milk. The ruminants consume a large volume of the feed of plant origin. They may contribute to the increased intake of POPs from the food of animal origin by humans (Costera et al., 2006). Through the lactation, the PCB levels in milk decrease by 25% and increase later during the lactation when the cows consume more silage (Thomas et al., 1999).

Pasture contamination in the industrialized areas may be the source of the long-term PCBs intake by dairy animals and their excretion to milk. Dairy animals have

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productive life much longer than meat-producing animals (La Rocca and Mantovani, 2006). Seven PCB congeners (PCB 28, 52, 101, 118, 138, 153, 180) are often found in the environment and human food in Europe and all around the world. Tremolada et al. (2014) reported that PCB absorption differs depending on the breeding conditions. If the cows are bred in the cowshed, the PCB absorption rate is higher than in the alpine conditions. They found the milk sum PCBs concentrations in the range of 0.75–1.33 ng g⁻¹ d.w. in Italian Alps. The results concerning milk analyses in Belgium reveal higher milk concentrations of PCB concentrations in high producing dairy cows (Petro et al., 2010). The dominant PCB congeners in whole milk from California were PCB-101, PCB-118, and PCB-138 (Chen et al., 2017). Among dioxin-like PCBs, 3,30,4,40,5-pentachlorobiphenyl (PCB 126) was the most important contributors to the toxic equivalency (TEQ) in cow's milk in Wallonia, Belgium (Focant et al., 2003). PCBs production in Slovakia, in the factory Chemko Strážske, located in eastern part of the country, was banned in 1984. However, Wimmerová et al. (2015) found that people living up to 70 km around the original source of PCB may be affected in this part of Slovakia. The congeners with the highest risk of exposure are PCB 52 and 153. Recent research shows that the area of about 235 km² may be contaminated (Strémy et al., 2019). The levels of PCBs and OCPs in the Netherlands, Norway and Slovakia have declined by 75% to 98% in the last 10 years, but in Slovakia, the highest PCBs and OCPs contamination is recorded. These chemical compounds

are present in the human organism at levels that may impair the vulnerable population subgroups (Čechová et al., 2017).

Except of animal milk, PCBs are transported also to human milk and can endanger the children's health. The study of Brajenović et al. (2018) concluded that the highest European levels of PCB were reported in the Czech Republic and Slovakia, most likely due to heavy industrial production. Authors also noted that the most abundant PCB congener in human milk is PCB 153. Milk is an important source of essential vitamins, minerals, proteins and other compounds for the human and especially child health. Therefore, the aim of this work was to analyze and compare the raw cow's milk, feed and soil samples from different areas of Slovakia based on the environmental regional classification.

2 Material and methods

2.1 Sample collection

The cow's milk, feed and soil samples were collected in three different areas of Slovakia based on the environmental regional classification (MESR and SEA, 2018) (Figure 1). Location around Novot' is considered as a region with undisturbed environment and it is located 95 km north-west of the former PCB production site (Chemko Strážske). The second analyzed area of Tulčík is located 45 km north-west of Chemko Strážske and it is considered as a region with a moderately

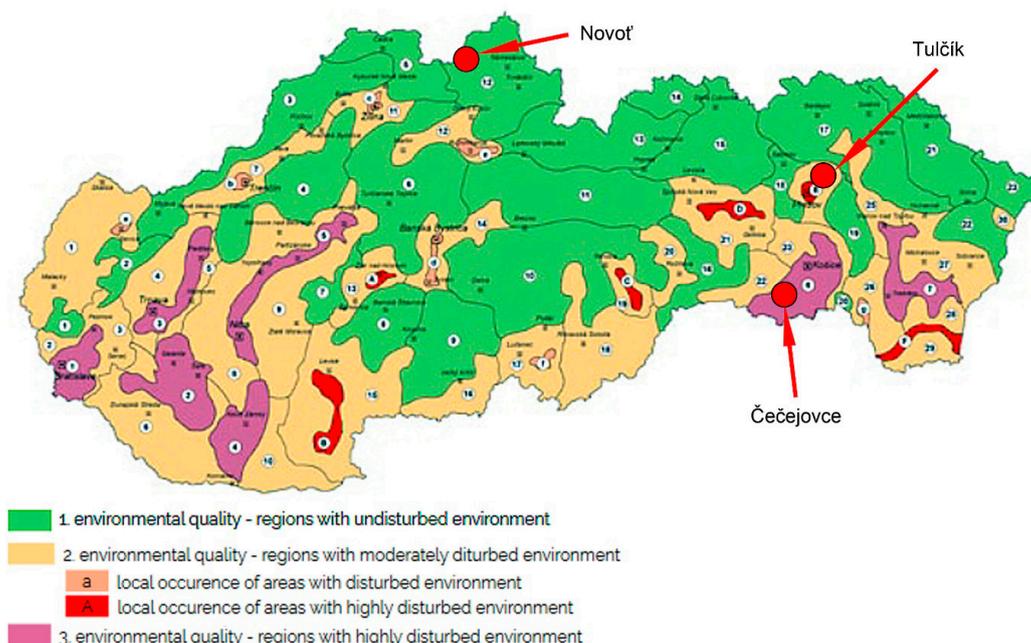


Figure 1 Environmental regional classification of Slovakia
Source: MESR and SEA, 2018
Three investigated regions (Novot', Tulčík, Čečejojce) are marked

disturbed environment, and the third area considered as a region with strongly disturbed environment is located around Čečejevce, 65 km south-west of the former PCB production site.

The pool milk samples were collected immediately after the milking two times a day, in the morning and in the afternoon. Samples were collected three times a year; in spring (April), in summer (July), and in autumn (September). The cows were milked using an automatic milking system and 500 ml of milk were collected during the two days. Two samples were collected from morning milking and two samples from afternoon milking. Five samples of milk were collected from each milking, in total 20 samples in spring, 20 samples in summer and 20 samples in autumn from each location. The total number of the dairy cows were as follows: Novot' area (220 cows; crossbreeds: Slovak spotted breed × Red Holstein breed), Tulčik area (450 cows; Slovak spotted breed), and Čečejevce area (340 cows; Black Holstein breed). Average milk samples from these cows were obtained from milk tanks immediately after the milking. Samples were kept in PET bottles at -18 °C until analysis.

Feed samples of total mixed ration (TMR) were collected in April ($n = 5$) and September ($n = 5$) from each observed location. The feed was made at all farms from local components. Samples were stored in plastic bags at -18 °C until analysis.

Five soil samples were collected from different places at each farm during the spring season (April). Samples were stored in plastic bags at -18 °C until analysis.

2.2 Sample analysis

The PCB congeners were analyzed in the samples as follows: milk and soil (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB-180), feed (PCB-101, PCB-138, PCB-153, PCB-180).

Milk samples were extracted with petroleum ether and diethyl ether mixture during the vigorous shaking. The extract was then filtered using sodium sulfate and the solvent was evaporated. The obtained fat diluted in the chloroform and purified by gel permeation chromatography (GPC). The PCB fraction was collected in the tube and the eluate with PCB was evaporated almost to dryness. The internal standard was then added and the solution was analyzed using the gas chromatography with electron capture detector method (GC-ECD) with HP Agilent 6890N with EC detector and automatic dozer (Agilent Technologies, USA).

Feed samples were extracted using acetone after adding the water in 2 : 1 ratio. After the liquid-liquid extraction, sodium chloride and the mixture of cyclohexane and

acetic acid ethyl ester were added. After the phases were separated, the organic phase was dried with sodium sulfate and concentrated. The equivalent volumes of ethyl acetate and cyclohexane were gradually added. Residual water was removed by the sodium sulfate and sodium chloride mixture and the solution was then filtered. The extract was purified by GPC and analyzed using gas and liquid chromatography (GLC) with Agilent 7890 B in combination with Agilent 7000 Triple Quad and Agilent 1260 Infinity II LC System in combination with Agilent 6400 QQQ LC/MS System (Agilent Technologies, USA).

The dry soil samples were extracted with n-hexane and acetone in ultrasonic bath. The extract was purified with concentrated sulfuric acid. The dried eluate was diluted in the n-hexane and analyzed using the GC-ECD method with HP 5890A with EC detector and automatic dozer (Agilent Technologies, USA). All samples were analyzed in certified laboratory Eurofins/Bel Novamann (Nové Zámky, Slovak Republic).

3 Results and discussion

The content of 7 PCB congeners in all raw milk samples was under the LOQ limit. Table 1 shows the results of analyses of cow's milk. There were no differences between the investigated areas. The quality of the environment by the environmental regional classification of Slovak Republic did not affect the milk PCB concentrations. Moreover, we did not record the effect of the season. The results were the same in spring (April), summer (July) and autumn (September). In contrast, Tremolada et al. (2014) found that the PCB levels in cow milk were season-dependent. The highest concentrations were recorded in autumn. The PCB absorption efficiency were lower at pasture than in the cowshed. An increasing levels of PCB in air were observed in summer season (Hao et al., 2018). The older analyses of the PCB exposure of the population in Slovak Republic showed decreasing trend from 1999 to 2003 and it has increased during 2004. In that period, the increased concentrations of PCB were found in eggs and milk samples (Šalgovičová and Pavlovičová, 2007). Milk samples of dairy cows in Belgium also exhibited low contamination levels. The milk from high producing cows is more risky because it can contain higher concentrations of chlorinated biphenyls. PCB in cow milk were recorded in the range of 1.8–35.3 ng g⁻¹ l.w. (Petro et al., 2010). Regarding the dioxin-like PCB content in pasteurized milk, analyses in Belgium revealed, that only PCB 81 was found in levels lower than the LOQ, but other important PCB congeners (77, 126, 169) were detected (Focant et al., 2003). Costera et al. (2006) found that concentrations of PCB congeners in goat milk fat decreased in the following order 153>138>118. The milk samples also contained PCB

Table 1 Concentration of PCB congeners in cow's milk in April, July and September in the investigated areas (mg kg⁻¹)

PCB congener	Area		
	undisturbed	moderately disturbed	strongly disturbed
PCB 28 – 2,2',4 – trichlorobiphenyl	<0.02	<0.02	<0.02
PCB 52 – 2,2',5,5' – tetrachlorobiphenyl	<0.02	<0.02	<0.02
PCB 101 – 2,2',4,5,5' – pentachlorobiphenyl	<0.02	<0.02	<0.02
PCB 118 – 2,3',4,4',5 – pentachlorobiphenyl	<0.02	<0.02	<0.02
PCB 138 – 2,2',3,4,4',5' – hexachlorobiphenyl	<0.02	<0.02	<0.02
PCB 153 – 2,2,4,4,5,5 – hexachlorobiphenyl	<0.02	<0.02	<0.02
PCB 180 – 2,2',3,4,4',5,5' – heptachlorobiphenyl	<0.02	<0.02	<0.02

0.02 mg kg⁻¹ = LOQ

Table 2 Concentration of PCB congeners in feed in April and September in the investigated areas (mg kg⁻¹)

PCB congener	Area		
	undisturbed	moderately disturbed	strongly disturbed
PCB 28 – 2,2',4 – trichlorobiphenyl	<0.05	<0.05	<0.05
PCB 52 – 2,2',5,5' – tetrachlorobiphenyl	<0.05	<0.05	<0.05
PCB 101 – 2,2',4,5,5' – pentachlorobiphenyl	<0.05	<0.05	<0.05
PCB 118 – 2,3',4,4',5 – pentachlorobiphenyl	<0.05	<0.05	<0.05
PCB 138 – 2,2',3,4,4',5' – hexachlorobiphenyl	<0.05	<0.05	<0.05
PCB 153 – 2,2,4,4,5,5 – hexachlorobiphenyl	<0.05	<0.05	<0.05
PCB 180 – 2,2',3,4,4',5,5' – heptachlorobiphenyl	<0.05	<0.05	<0.05

0.05 mg kg⁻¹ = LOQ

180 which is the pentachlorinated congener. Aydin et al. (2019) found the minimum, the maximum, and the mean total PCBs concentrations 10.59 ng g⁻¹ l.w., 35.91 ng g⁻¹ l.w., and 24.41 ng g⁻¹ l.w. in the raw milk samples in Turkey, respectively. The results of Ahmadkhaniha et al. (2017) revealed that six dioxin-like PCBs were found in all milk samples in Iran with the mean concentration of 18.92 ng g⁻¹ fat. All of milk samples were compliant with EU established provisional value of 40 ng g⁻¹ fat. The sum of PCB congeners was lower than 3 pg g⁻¹ fat which is the limit set for cow's milk. The maximum levels of PCB in raw milk is set to 5.5 pg g⁻¹ (sum of dioxin-like PCBs) and 40.0 ng g⁻¹ (sum of PCB 28, 52, 101, 138, 153, 180) by the Commission Regulation (EU) No 1259/2011 (Commission Regulation EU, 2011). Serpe et al. (2015) reported that all dairy products (cow, buffalo and ewe milk) from 50 farms in Campania Region, Italy, were in accordance with the maximum levels set by EU in Regulation 1259/2011, but the limits reported in EU Recommendations 516/2011 (Commission recommendation, 2011) and 711/2013 (Commission Recommendation, 2013) were exceeded in two sheep farms and two buffalo farms. The concentrations of 12 dioxin-like PCBs in bovine milk were 0.49–0.56 pg WHO-TEQ g⁻¹ fat. Later, in the same region of Italy, Chirollo

et al. (2018) found the levels of dioxin-like PCBs 8.33 to 13.95 pg g⁻¹ fat in dairy buffaloes (*Bubalus bubalis*) milk, dominated by congeners PCB 126 and PCB 169.

In our analyses, all PCB congeners in all milk samples were under the LOQ (0.02 mg kg⁻¹). The fact that the most important PCBs were not found in any sample even in the area marked as moderately disturbed environment (around Tulčík) and region with strongly disturbed environment (around Čečejevce), which are close to the former PCBs production site, indicates the safer environment for the production of food of animal origin, like milk, and also for the human health than in the past. Anyway, the fatty food is considered to be the main source of human exposure to PCB but it is important to monitor the animal food sources like meat, milk and eggs (Wimmerová et al., 2015). In this context, foods of animal origin have higher bioaccessibilities than food of plant origin. Bioaccessibility of PCB from milk powder is relatively high and reaches 28.4% (Shen et al., 2016).

The feed PCB concentrations were under the LOQ limit (0.05 mg kg⁻¹) in our research (Table 2). This situation was the same in the spring and also in the autumn. Feed seems to be a major route for PCB exposure of animals.

Table 3 Concentration of PCB congeners in soil in April in the investigated areas (mg kg⁻¹)

PCB congener	Area		
	undisturbed	moderately disturbed	strongly disturbed
PCB 28 – 2,2',4 – trichlorobiphenyl	<0.0001	<0.0001	<0.0001
PCB 52 – 2,2',5,5' – tetrachlorobiphenyl	<0.0001	<0.0001	<0.0001
PCB 101 – 2,2',4,5,5' – pentachlorobiphenyl	<0.0001	<0.0001	<0.0001
PCB 118 – 2,3',4,4,5 – pentachlorobiphenyl	<0.0001	<0.0001	<0.0001
PCB 138 – 2,2',3,4,4',5' – hexachlorobiphenyl	<0.0001	<0.0001	<0.0001
PCB 153 – 2,2,4,4,5,5 – hexachlorobiphenyl	<0.0001	<0.0001	<0.0001
PCB 180 – 2,2',3,4,4',5,5' – heptachlorobiphenyl	<0.0001	<0.0001	<0.0001

0.0001 mg kg⁻¹ = LOQ

An interesting findings concerning the similar OCP compounds (dioxins and furans) published Fürst et al. (1993), that the transfer of these compounds from air through the grass to cow is more significant than from the soil. The authors stated that the accumulation of these contaminants depends on the congeners and therefore, it is inappropriate to set the maximum limits for soil or grass contamination based on toxic equivalents in order to reduce the concentrations of polychlorinated dioxins and furans in cow milk. Also Weber et al. (2018) confirm that the PCBs highly affect the plants and feed for the livestock from the atmosphere. Authors warn, that soil PCB concentrations of 2–5 ng TEQ kg⁻¹ can cause the increase of the PCBs in meat and eggs over the EU limits. Rychen et al. (2014) warn that ruminants may be exposed to contaminated soil and feed and therefore, it is important to determine the availability of polychlorinated compounds from each of these matrices. In this sense, we have analyzed also the soil and feed samples beside the milk. Almost 3-times higher total TEQ was determined in a cows' milk sample from a farm at a village in the Krompachy region than a maximum level of 6 pg g⁻¹ fat set by the European Commission. The second highest levels of dioxin-like PCBs were observed in the Nemecká region. The total toxic equivalent in cows' milk ranged from 0.59 to 16, in goats' milk from 0.88 to 33 and in ewes' milk from 0.61 to 20 pg g⁻¹ fat (Čonka et al., 2015). It is interesting, that we were not able to find any PCB congener in the soil in all investigated areas, no matter their location. Table 3 shows the results of the soil analyses. The concentration of all 7 PCB congeners were under the LOQ (0.0001 mg kg⁻¹) also in the regions close to the former PCB production site.

Anyway, Rychen et al. (2014) noted that the polychlorinated contaminants are able to travel to distant areas so the source of contamination may not be close to the affected vegetation. The levels that are proposed to be safe, are exceeded around the sources of PCBs. However,

the soil PCB concentrations even in rural areas may be safe for food production if there is not present the source of contamination (Weber et al., 2019). In our study, the milk concentrations under the LOQ reflect the situation for the soil and feed from the specified areas of Slovak Republic. Costera et al. (2006) calculated the carry-over rate for PCB congeners. The highest values were found for PCB congeners 105, 118 and 157, while the lowest transfer rates were recorded for PCB congeners 77 and 123. Authors suggested that the transfer of the PCBs is related to the number of chlorine substituted atoms. The highest values were found for penta-, hexa- and heptachlorinated congeners (PCB 105, 118, 157, 156, 114, 189). The lowest transfer rate was found for congeners with 3 to 5 chlorine atoms (PCB 28, 52, 77, 101).

4 Conclusions

Animal products like milk, milk products and others are the main contributors to human exposure to PCBs and other OCPs. In our study, all samples of raw milk, feed and soil were under the LOQ and did not exceed the limits set by European Commission in all investigated regions, also in the area considered as a region with strongly disturbed environment in Eastern Slovakia around Čečejevce. Despite the results indicate the decreasing trend in PCBs occurrence in the environment and food sources, there is a constant need to monitor environmental burden of PCBs in the different regions of Slovakia and in food sources.

Acknowledgments

This publication was supported by Operational Programme Research and Innovation for the project: Supporting the research activities in AgroBioTech, 313011T465, co-funded by European regional development fund.

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