



PRELIMINARY DATA ON POLYCHLORINATED BIPHENYLS (PCBs) IN COWS' MILK FROM BOSNIA AND HERZEGOVINA MARKET

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ABSTRACT: In this work, both farm milk samples (n=3), as well as samples of milk from Bosnia and Herzegovina market (n=7), were analyzed to quantify the presence of polychlorinated biphenyls. The PCB content (as Aroclor 1254) was determined in the fat extracts using the PCB ELISA kit, according to the procedure provided by the manufacturer.

The total PCB concentrations (as Aroclor 1254) in individual milk sample ranged from <LoD to 787.74 µg/kg lipid weight (l.w.). The calculated Σ₆PCB were ranged from <LoD to 187.72 µg/kg l.w.

In one out of ten analyzed samples the presence of PCB higher than maximum limit (ML) was found. However, the absorbance measured for this sample is out of the linear range. In this case the sample can be considered as a suspect and should be analyzed with the quantitative confirmatory method.

In other milk samples the concentration of PCB was in the acceptable range, and far below the ML.

Key words: *polychlorinated byphenils, Aroclor1254, ELISA, milk*

INTRODUCTION

Consumption of cow's milk has long tradition in human nutrition (Haug, 2007). Milk is a very important source of dietary energy, protein and fat. Cow milk contains high-quality proteins, with good balance of all the essential amino acids (FAO, 2013). Proteins have multiple roles in the body (e.g. to form antibodies, to build and repair body tissues etc.). Cow's milk also contains calcium, phosphorus, magnesium, and potassium and it is a significant source of vitamins B, A and D. The U.S. government's Dietary Guidelines suggest the following daily consumption of milk: for children 1-8 years old, 2 servings (one serving is 125 ml), children older than 9 years and adults 3 servings (IDFA, 2014).

Polychlorinated biphenyls (PCBs) make up a highly toxic group of pollutants that are known to persist in the environment, accumulate in lipids, tend to biomagnify through the food chain, and have the potential to cause adverse health effects (Djedibegović, 2015). Currently, the major sources of PCBs are their environmental deposits from past releases; hence, volatilization from land and surface water is an important source for the global distribution of PCBs (ATSDR, 2000). Generally, fatty foods of animal origin are the major sources of human exposure to lipophilic contaminants (Ahmadkhaniha, 2017). Previous studies have shown that the main route of human exposure to PCB is contaminated

food. Milk is one of the main constituent of the daily diet. Bosnia and Herzegovina (BiH) is, according to the GEMS World Health Organization (FAO/WHO, 2012), the cluster with the highest uptake of milk per capita (456.5 g/day). Thus, knowledge of cow's milk contamination by PCBs provides important information about human exposure to these contaminants, through the ingestion of dairy products.

MATERIALS AND METHODS

Chemicals and reagents

Cyclohexane, methanol and 2-propanol (all analytical grade), decane (purum), n-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). PCB standard (Aroclor 1254) was purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany).

Nitrogen 5.0 was supplied from Messer (Sarajevo, BiH). Ultrapure water (>18mΩ) was supplied by Arium® mini Lab Water System (Goettingen Germany). PCB standard (PCB30) was purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany).

Sample collection and treatment

Cow's milk samples from three different farms in Bosnia and Herzegovina were collected in May 2014 and commercial packed pasteurised milk was purchased in Sarajevo (Bosnia and Herzegovina) (7 samples). Samples from farms (50 ml) and from market (0,5 L) were transported to the laboratory, labelled (F1-F3 samples from farms and T1-T7 samples from market) and stored at -20 °C until analysis.

From each collected sample, an aliquot of 11 mL were treated with sulfuric acid (10 mL) and amyl alcohol (1 mL), carefully mixed and centrifuged at 1200 rpm for 5 minutes (IEC CL-10 Centrifuge, Thermo Fisher Scientific Inc., USA). The upper layers were transferred into clean test tubes, dried (36 °C) in a stream of nitrogen and the fat fraction of milk was weighted.

To each of the measured aliquot of fat 2 mL of cyclohexane and 50 µL of the internal standard (PCB30) were added and mixed on a vortex (Vortex-Genie 2, Scientific Industries, NY, USA).

The obtained mixture was cleaned with a concentrated sulfuric acid as described for fish and shellfish fat (Omeragic et al, 2016; Djedjibegovic et al 2015). Purified mixture was evaporated using nitrogen and dissolved in aqueous methanol (1:1 v/v).

Determination of PCBs

The PCB content (as Aroclor 1254) was determined in the fat extracts using the PCB ELISA kit (Abraxis/Biosense PCBs ELISA kit, Warminster, PA, USA), according to the procedure provided by the manufacturer. The spectrophotometric quantification was performed at 450 nm using RPA-I RaPID Analyzer (spectrophotometer, Strategic Diagnostics Inc, Newark, DE USA). The calibration curve was constructed with Aroclor 1254 standard solutions (0.25, 1.5, 2.5, 5.0, 10.0 and 25.0 ng/mL). The results were calculated as the mean of three measurements, using logit transformation (equation 1) (Bolton, 1997).

$$\text{logit} A/A_0 = \log \left(\frac{\frac{A}{A_0}}{1 - \frac{A}{A_0}} \right) \quad [1]$$

where A is mean absorbance value for standard/sample and A₀ is a mean absorbance value for negative control.

Calculation

For the method limits of detection (LoD) and limits of quantification (LoQ), a conservative estimate of SD was taken from the standard deviation of measurements of a concentration T6 sample. The method limits of detection and limits of quantification were calculated using equations 2 and 3, respectively.

$$\text{LoD} = 3 * \text{SD} \quad [2]$$

$$\text{LoQ} = 10 * \text{SD} \quad [3]$$

The resolution of the ELISA test (AC) was calculated using equation 4:

$$\text{AC} = 2 \times \sqrt{2 \times \text{SD}} \quad [4]$$

where SD is the standard deviation at the concentration of interest (Richter, 1994).

As a concentration of interest, concentration corresponding to 95 percent of the distribution of Σ_6 PCB concentrations found in milk samples in European countries (EFSA, 2012) was defined, and it was 16 $\mu\text{g}/\text{kg}$ of fat. The SD of the measurements at the PCB level detected in T1 sample and calculated as Σ_6 PCB concentrations (12.22 $\mu\text{g}/\text{kg}$ on a lipid basis (l.w.)) was used as the concentration of interest.

Recovery factor was estimated by analysing positive control using equation 5:

$$R_s = \frac{C_s}{C_k} * 100 \quad [5]$$

where C_s is the measured concentration and C_k expected concentration of positive control (5 ng/mL).

The positive and negative control were provided in the commercial PCB ELISA kit. Positive control contained 5 ng/mL of PCB (as Aroclor 1254) in methanol solution containing preservative and stabilizers and negative control was a methanol solution containing preservative and stabilizers without any detectable PCB.

Concentrations of PCBs (as Aroclor 1254) are presented as $\mu\text{g}/\text{L}$ ($\mu\text{g}/\text{L}$ w.w.) of native sample and $\mu\text{g}/\text{kg}$ on a lipid basis ($\mu\text{g}/\text{kg}$ l.w.).

RESULTS AND DISCUSSION

Linearity of the ELISA test for PCBs determination in milk samples was in the range of 0.25–25.0 ng/mL ($R^2 = 0.9804$) (Figure 1). The limit of detection (LoD) was 0.19 $\mu\text{g}/\text{L}$ w.w. and the limit of quantification (LoQ) was 0.63 $\mu\text{g}/\text{L}$ w.w.

The recovery for the positive control was 96.2%. The resolution of the ELISA test was 0.13 $\mu\text{g}/\text{l}$ w.w. (8.50 $\mu\text{g}/\text{kg}$ l.w.) at 0.33 $\mu\text{g}/\text{l}$ w.w. (12.21 $\mu\text{g}/\text{kg}$ l.w.)

The presence of PCBs was not detected in the blank indicating the absence of subsequent contamination of samples during preparation.

The requirement for analytical method performances in food safety control is LoQ less than one-fifth of the maximum level (ML) for the PCB concentration (EFSA,

2012). The ML of Σ_6 PCBs in milk is 40 $\mu\text{g}/\text{kg}$ l.w. (EC, 2011), and the LoQ analytical methods should be less than 8 $\mu\text{g}/\text{kg}$ l.w., which was achieved by the ELISA test in our work. The ELISA test performances obtained in our work were acceptable in terms of accuracy.

The test resolution shows that we were able to statistically discriminate concentration of interest (16 $\mu\text{g}/\text{kg}$ l.w.) from permitted maximum level set by European Commission (EFSA, 2012) and this test is acceptable for screening purposes.

PCBs can be analyzed and quantified as Aroclor mixtures or as individual congeners. Permitted maximum levels of the PCBs in food are set as sum of specific number of congeners.

PCB data in this paper are presented as equivalents of Aroclor 1254. These data cannot be directly compared to permitted maximum levels set by European Commission.

Since each Aroclor mixture has known PCB congener composition, we multiplied our results by sum of weight percents of six indicator congeners (0.19, 5.38, 8.02, 5.80, 3.71, 0.67 for PCB 28, 52, 101, 138, 153 and 180, respectively) in Aroclor 1254 mixture (Frame, 1996).

The PCB concentrations in milk samples are summarised in Table 1. The total PCB concentrations (as Aroclor 1254) in individual milk sample ranged from <LoD to 787.74 $\mu\text{g}/\text{kg}$ l.w.. The calculated Σ_6 PCB were ranged from <LoD to 187.72 $\mu\text{g}/\text{kg}$ l.w.

With the exception of sample T6, PCBs concentrations found in all other samples were greater than LoQ. The PCBs content in milk fat varied for factor 4. In sample F1 the PCBs concentration was at least one order of magnitude greater than that found in other samples (Table 1). At the same time, this is the only sample in which the concentration of PCB was greater than ML (40 $\mu\text{g}/\text{kg}$ l.w.), while concentrations in other samples were at least three times lower than ML. In the inspection of the results it was noted that the measured absorbance of F1 sample was significantly outside the linear range, and this sample could be described as suspect.

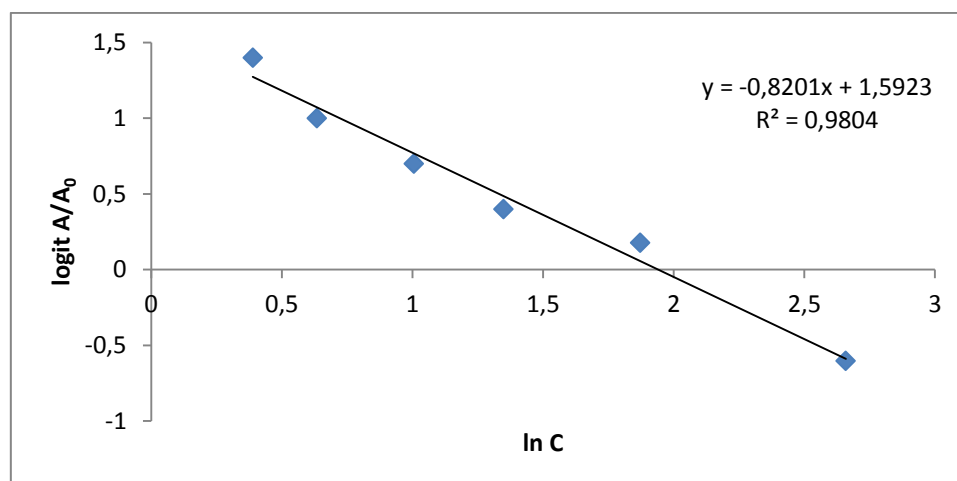


Figure 1. The standard calibration curve based on Aroclor 1254

Table 1.
The PCB concentrations in milk samples

Sample ID	Aroclor1254			Σ_6 PCB	
	PCB _{ELISA} ($\mu\text{g/L}$)	PCB _{w.w.} ($\mu\text{g/L}$)	PCB _{l.w.} ($\mu\text{g/kg}$)	PCB _{w.w.} ($\mu\text{g/L}$)	PCB _{l.w.} ($\mu\text{g/kg}$)
Positive control	4.81**	-	-	-	-
T1	15.27	1.39	51.22	0.33	12.21
T2	11.48	1.04	33.77	0.25	8.05
T3	11.48	1.04	42.60	0.25	10.15
T4	10.23	0.93	30.99	0.22	7.38
T5	15.89	1.44	47.52	0.34	11.32
T6	3.30	0.30	13.46	0.07	3.21
T7	ND	ND	ND	ND	ND
F1	209.70	19.06	787.74	4.54	187.72
F2	8.60	0.78	50.46	0.19	12.02
F3	7.53	0.68	45.62	0.16	10.87
Blank	ND	ND	ND	ND	ND

T-milk samples from market; *F*- milk samples from farms;

**Recovery for the positive control is 96.2%; ND – not detected, i.e. <LoD

According to the EFSA Report (EFSA, 2010), the mean of Σ_6 PCB in 5640 collected milk products in 18 European countries, Iceland and Norway, was $9.2 \pm 3.4 \mu\text{g/kg l. w.}$ (median $9.0 \mu\text{g/kg l. w.}$).

The average concentration of Σ_6 PCB in milk samples analyzed in this paper was $26.29 \pm 56.86 \mu\text{g/kg l. w.}$, with evident influence of extreme F1 sample (median $10.51 \mu\text{g/kg l. w.}$). By omitting the F1 sample, the average content of Σ_6 PCB in our samples was $8.36 \pm 4.24 \mu\text{g/kg l. w.}$ (median $10.15 \mu\text{g/kg l. w.}$).

In conclusion, levels of Σ_6 PCB in our samples generally do not deviate from the European samples.

The F1 sample contains a significantly higher level of Σ_6 PCB. The cause of high level of PCB in this sample could be a result of residue accumulation in the tissues following the cattle's exposure from feeding on contaminated feedstocks or from drinking contaminated water (Gomes Martins, et al., 2013).

Bernard et al. (2002) found a concentration of six indicator PCBs up to $160 \mu\text{g/kg l. w.}$ in milk originally from cows fed with contaminated food. Turrio-Baldassarri et al. (2009) found PCBs residues in concentrations ranging from $183\text{--}477 \mu\text{g/kg l. w.}$ in milk samples originally from farm located near the factory for PCB production. PCB mixtures were never manu-

factured in BiH, nor was there any production of equipment containing PCBs, but during the last civil war in BiH, large amounts of persistent organic pollutants were generated and emitted into the environment as a result of partial or complete destruction of industrial facilities, military targets, infrastructure, explosions and uncontrolled fires (Klánová et al. 2007).

Effects of non-dioxin-like PCBs (ndl-PCBs) on human health are still not sufficiently explored to make it possible to assess the risks to human health. Therefore, the risk is further estimated based on the sum of dioxin-like PCBs (dl-PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), expressed as pg toxic equivalent (TEQ) in g fat.

This concentration can be calculated with sufficient accuracy only if the concentrations of each PCB congener, PCDD and PCDF are known. Unlike six indicator PCBs, dl-PCBs, PCDDs, and PCDFs show some variation in content in individual PCBs mixture, so TEQ content in individual Ar1254 series varied for one order of magnitude (17-392 pg TEQ/g Ar11254) (EFSA, 2005). Considering significant uncertainty, this calculation was not used in the paper. However, in the EFSA report, correlation of content of Σ_6 PCB in analyzed milk samples (n=213) and total TEQ_{WHO05} was described by regression equation: ndl-PCB=10.91 dl-PCB+1.88 (EFSA, 2012). Using this equation the calculated TEQ_{WHO05} value for our samples would be 0.00-0.95 pg/g fat (except for sample F1: 17.03 pg/g of fat), which is significantly below ML of 5.5 pg TEQ_{WHO05}/g of fat (EC, 2011).

CONCLUSIONS

The analytical performance of the commercial ELISA kit allows its application as a screening method for determining PCBs in milk.

The presence of PCBs was detected in 9/10 of analyzed milk samples. One out of ten samples was suspected to contain high levels of PCBs. This sample originated from a farm and it is possible to reflect the elevated contamination of forage,

which should be further examined. Generally, this preliminary study suggested that PCB concentrations found in milk samples from the BiH market, do not indicate an unacceptable health risk to consumers.

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САДРЖАЈ ПОЛИХЛОРОВАНИХ БИФЕНИЛА У МЛЕКУ СА БИХ ТРЖИШТА

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Сажетак: У овом раду су анализирани узорци млека са фарми (n=3), те узорци из комерцијалних извора (n=7) у Босни и Херцеговини (БиХ) на присуство полихлорованих бифенила (ПХБ). Садржај ПХБ (као Арохлор 1254) је одређен у екстракту масти кориштењем ПХБ ЕЛИСА кит, према процедури предложеној од стране произвођача.

Садржај укупних ПХБ (изражен као Арохлор 1254) у појединим узорцима млека се кретао у распону <LoD to 787.74 µg/kg l.w. Израчунати садржај Σ₆ПХБ био је у распону <LoD to 187.72 µg/kg l.w. У једном од десет анализираних узорака нађена концентрација ПХБ је била већа од максимално дозвољене концентрације (МДК), међутим, измерена апсорбанца за овај узорак налази се изван линеарног распона, тако да се овај узорак може сматрати суспектним, што би требало проверити референтном квантитативном методом.

У осталим узорцима млека, концентрације ПХБ су биле у прихватљивим границама, и далеко од максимално дозвољене концентрације.

Кључне речи: полихлоровани бифенили, Aroclor1254, ELISA, млеко

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