



LEVELS OF INDICATOR POLYCHLORINATED BIPHENYLS IN HUMAN MILK FROM VARNA REGION, BULGARIA

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ABSTRACT:

Human milk is an appropriate tool for the assessment of the body burden of humans with lipophilic persistent organic pollutants such as polychlorinated biphenyls (PCBs).

Purpose: The aims of the study were to determine the levels of polychlorinated biphenyls in the breast milk of women living in Varna, Bulgaria and to evaluate the association with demographic characteristics.

Material and methods: Breast milk samples were collected in 2019 - 2020 from 28 healthy primiparae mothers. The six indicator PCB congeners were determined in individual mother's milk samples by capillary gas chromatography system with mass spectrometry detection (GC-MS).

Results: The predominant PCB congener measured in human milk was PCB 153, followed by PCB 138 and PCB 180. The lower chlorinated congeners PCBs (28, 52 and 101) were found below the limit of detection in most individual samples. The median level of the Indicator PCBs in milk samples was 29.1 ng/g lipid weight. The highest PCBs levels were found in breast milk from primiparae mothers 36 – 40 age (36.8 ng/g lipid as a sum of six Indicator PCBs), suggesting bioaccumulation of these pollutants.

Conclusions: Positive association was found between the Indicator PCBs levels and the age of mothers. The levels of the Indicator PCBs in breast milk were found to be lower than levels reported in other studies in the European countries.

Keywords: Breast milk, Polychlorinated biphenyls, Bulgaria,

INTRODUCTION

Polychlorinated biphenyls (PCBs) are widespread pollutants in the environment and are persistent in physicochemical and biological degradation. These compounds have a tendency to accumulate in organisms and are magnified through the food chain [1, 2]. The experimental studies by animals found adverse effects caused by PCBs, such as reductions in immune system function, endocrine disruptions and impaired reproduction [3]. The International Agency for Research on Cancer (IARC) and U.S. Environ-

mental Protection Agency (US EPA) have determined PCBs as probably carcinogenic to humans (group 2A and 2B) [3]. PCBs mostly accumulate in adipose tissue in the human organism, in blood serum and human milk [4]. In recent years, a number of epidemiological studies have investigated the relationship of PCB exposure to breast cancer risk [5]. Analysis of the data showed that some individual PCB congeners might influence on the development of human breast cancer [5].

Breast milk contains nutrients and protective immunological components that have a positive effect on infant health, nevertheless may also contain lipid-soluble and persistent contaminants such as PCBs [2]. Determination of PCBs in breast milk is a non-invasive and important method for objective assessment at the exposure levels to humans [6]. The levels of persistent organic pollutants in breast milk largely reflect the amount of contaminants in the body of mothers [1, 2, 6]. However, the available scientific data do not provide conclusive evidence of clinically relevant adverse health effects on infants exposed to environmental chemicals in breast milk [7, 8]. The scientific studies showed, in most cases, that the benefits of breast-feeding outweigh any risks from exposure to PCBs [2, 9].

There are limited data on levels of persistent organic pollutants (POPs) in the breast milk of Bulgarian women. To our knowledge, only one study was conducted as part of 2000–2003 WHO POPs global exposure survey on human milk [9]. The aims of the study were to determine the levels of polychlorinated biphenyls in the breast milk of women living in Varna, Bulgaria and to evaluate the association with demographic characteristics.

MATERIALS AND METHODS:

Sampling collection

The organization of sampling and individual interviews of donors was conducted according to the World Health Organization's (WHO) protocol and was approved by the local committee of medical ethics at the Medical University of Varna (protocol No. 85/26.07.2019) in accordance to the Declaration of Helsinki.

Samples were collected from primiparae mothers af-

ter the fourth and until a twelfth week after delivery in the period October 2019 – December 2020. The mothers were informed about the objectives of the study and agreed to voluntary participation by a signed declaration of informed consent. Data on maternal age (range, 25–40 years), body weight and height, place of living, and dietary habits were collected by “face to face” questionnaire. The collected milk samples were stored at -18°C until the laboratory analysis.

Chemical analysis

PCBs were analyzed in human breast milk following the analytical protocol based on European standardized methods EN 1528-1996 with some modifications. The milk samples were defrosted, and next were slowly warmed to 36°C and carefully homogenized. Ten grams of breast milk were weighed in a 50 mL glass centrifuge tube, and the sample was spiked with internal standards (PCB 30 and PCB 204, Dr Ehrenstorfer Laboratory). Lipid extraction was performed by three-step liquid-liquid extraction using solvent mixture of hexane: acetone in different ratio 1:0 v/v, 2:1 v/v, 1:1 v/v, respectively. The hexane layers were collected and filtered on anhydrous sodium sulfate and evaporated to dryness in a rotary vacuum evaporator. Lipid content was determined by the gravimetric method. The extract was cleaned-up on a self-packed multilayer glass column filled with sodium sulfate, 2 g of neutral silica (60–230 mesh), 2 g acid silica, 1 g neutral silica and anhydrous sodium sulfate from bottom to top, respectively. PCBs were eluted with 10 ml n-hexane followed by 20 mL n-hexane/dichloromethane (9:1 v/v). The eluates were concentrated to near dryness and reconstituted in 0.5 mL in hexane.

The quantitative analysis of PCBs was carried out on a gas chromatograph (GC/MS) GC FOCUS (Thermo Electron Corporation, USA) equipped with POLARIS Q Ion Trap mass spectrometer and an AI 3000 autosampler. The chromatographic separation of PCB congeners was performed in split less mode using a TR-5ms capillary column coated with 5% phenyl methyl siloxane (length of 30 m, 0.25 mm I.D. and a film thickness of 0.25 μm). The column temperature was programmed as follows: 60°C for 1 min, then 30°C/min to 180°C, 2°C/min to 260°C, 30°C/min to 290°C with a final hold for 2.0 min. Helium was applied as carrier gas at a flow rate of 1 ml/min.

Table 1. Concentrations of Indicator PCB congeners (median and range expressed in ng/g lipid weight) in breast milk (n=28), nd – not detected, n - number of samples

Compound	Number of positive samples > LOQ, n	Positive samples % > LOQ	Median	Range
PCB 28	6	21	0	nd - 2.76
PCB 52	1	4	0	-
PCB 101	4	16	0	nd - 2.00
PCB 153	28	100	15.18	4.87 - 35.07
PCB 138	28	100	7.91	0.87 – 18.29
PCB 180	24	86	3.83	1.40 – 15.99
Sum Indicator PCBs, ng/g lw	28	100	29.11	5.74 – 66.14

Pure reference standard solutions (PCB Mix 20 - Dr Ehrenstorfer Laboratory) were used for instrument calibration, recovery determination and quantification of compounds. Six PCB congeners (IUPAC No. 28, 52, 101, 105, 138, 153, 180) were analyzed in prepared extracts. Each sample was analyzed three times and was taken an average of the results obtained. The limits of quantitation (LOQ) for the components determined on the G.C.–M.S. varied for individual PCB congeners from 0.21 to 0.36 ng/lipid weight (lw). The quality control was performed by analysis of certified reference material: BCR450 (PCBs in milk) – Institute for Reference Materials and Measurements, European commission. In each analysis batch, procedure blank (Milli-Q water) was included.

Statistical methods

The statistical differences between the mean values of the data were evaluated by a Student t-test, and a significance level of $p < 0.05$ was used. All statistical calculations were made by the SPSS V19.0 software package for Windows (SPSS Inc., Chicago, IL, USA). For PCBs, whenever the residue value was below the limit of detection ($< LOD$), the zero value was used.

RESULTS

The characteristics of women who participated in the present study (n=28) are summarized in detail. The age of selected mothers was between 25 and 40 years (mean 31 years), and the mean body mass index (BMI) was 21.3 kg/m² (ranged 17.5 – 31.9 kg/m²). Twenty-eight milk samples were collected from the Varna region in the period October 2019 – December 2020, and participants were grouped by age: group 25-30 years (15 donors); group 31-35 years (8 donors) ; age 36-40 years (5 donors).

Lipid percentage in milk samples was in the range of 1.5 to 6.3 % (mean of 3.4%). The levels of six Indicator polychlorinated biphenyls (I-PCB) congeners were measured in human milk collected from 28 mothers living in the Varna region, Bulgaria. PCB congeners 138, 153 and 180 were the major congeners measured in all milk samples. The median concentration of sum Indicator PCBs in breast milk was 29.1 ng/g lw. Data of Indicator PCB congeners found in the milk samples are summarized in Table 1 as median and range in positive samples ($>LOQ$).

DISCUSSION:

Levels of PCBs in human milk

The concentration order of the most abundant congeners was as follows: PCB 153 > PCB 138 > PCB 180. The lower chlorinated congeners PCB 28, PCB 52, and PCB 101 were found in levels close to the LOD for most of the human milk samples. A similar pattern with PCB 153 as the predominant congener rather than PCB 138 and 180 was observed in human milk samples from Italy [6, 10], Northern and Southern Norway [11] and Croatia [12]. The bioaccumulation of congeners with high degrees of chlorination in the biphenyl rings is a typical characteristic of PCBs [13]. The sum of PCB 138, 153 and 180 accounted for 93.7% of the total PCB levels. The total sum of Indicator PCBs found in the milk samples of mothers from the Varna region was 29.11 ng/g lipid (as median value). The results obtained were lower than PCB levels in a previous study (42 ng/g lipid) carried out on human milk pools of Bulgarian mothers from Sofia and Bankja in 2000 – 2003 [9].

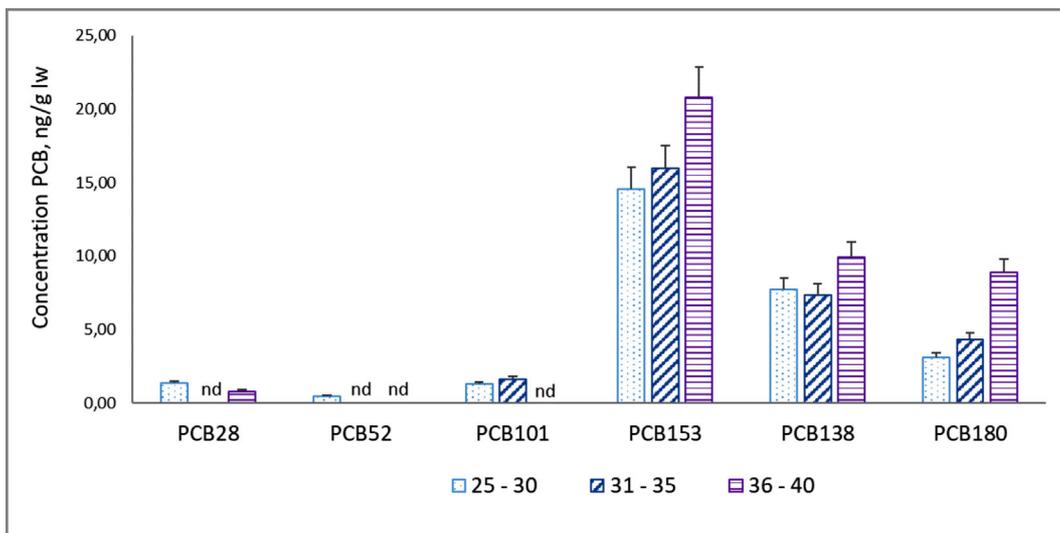
The median sum of indicator PCBs (PCB 28, 52, 101, 138, 153 and 180) in breast milk observed in our study (29.1 ng/g lw) was found to be lower than the values measured in other European countries: in Poland - 92 ng/g lw [1]; in Norway human milk - 115 ng/g lw

[11]; in Italy (Roma 2007) – 117 ng/g lw and in France (2007) - 203 ng/g lw [4]. Our results are lower or comparable to concentrations of PCB indicators in human milk from some Balkan countries: Greece – 94.4 ng/g lw; Croatia (Zadar, 2011) – 27.5 ng/g lw; Hungarian - 34 ng/g lw [4, 14].

Factors associated with PCBs body load

We investigated the possible correlation between the levels of PCB compounds in the samples and the age of mothers. The data analysis showed a strong positive correlation between the mother's age and levels of Indicator PCBs (Fig. 1), suggesting that body load for these pollutants was age dependent. The highest PCBs levels were found in breast milk from primiparae mothers 36 – 40 age (median 36.8 ng/g lipid as a sum of six Indicator PCBs), assuming bioaccumulation of these pollutants. The data analysis showed maternal age was the most important determinant of levels of PCBs in human milk among primiparae mothers, similar to recent studies undertaken in Norway and Belgium [2, 15]. A positive association between PCBs levels and age has been well established in other studies [16, 17, 18], and observations in our study showed a similar pattern.

Fig. 1. Distribution pattern of Indicator PCBs (ng/g lw) in breast milk by age groups of the study population



Most mothers (71.4%) have a healthy body mass index (in the range of 18.5–24.9) before delivery, 21% have BMI below 18.5. The data analysis showed no statistically significant relationship between BMI and PCB levels in milk samples. Only two participants had a BMI > 25 (7%), and we did not make any appreciable conclusions when compared to the BMI of 18.5–24.9 group. The body mass index pattern by the age groups did not show any significant differences.

The most important way of human exposure to PCB is the consumption of animal-origin foods, especially fish and fish products, which account for approximately 80% of the overall exposure [17, 19]. In our study population,

only a few participants consumed fish very frequently (2x/week; 7.1%). The majority of the participants (over 92%) consumed less fish and fish products than recommendations (< 2x/week). Less than half of mothers consumed fish once a week (42.9%). In our study, we did not find any positive correlation between fish consumption and levels of PCBs in breast milk. Thus, this may be related to the different dietary habits of milk donors.

The low levels of PCBs observed in breast milk samples correspond with the fact that no industrial production of PCBs took place in Bulgaria. Our previous studies on levels of PCBs in fish and seafood from the Bulgarian Black Sea coast showed lower levels of these

pollutants compared to other seas [19, 20]. Even higher consumption of local fish species would not pose a significant risk to human health.

Further investigation of POPs levels in breast milk will focus on studying other potential organochlorine contaminants in a larger group, including mothers with more children, and assessing potential exposure.

CONCLUSIONS

The study presents actual data on PCBs levels in the breast milk of mothers living in Varna, Bulgaria and shows that the body load of these pollutants was age dependent. The concentrations of PCBs determined in this study were found to be lower than data available from a previous study carried out in 2000 - 2003 of Bulgarian mothers. In general, results in PCBs levels found in the

breast milk of mothers from Varna, Bulgaria, were found to be lower than concentrations measured in European studies. We can conclude that the levels of PCBs in mother's milk are very low, and the benefits of breastfeeding far outweigh any toxicological disadvantages.

Abbreviations:

PCBs - polychlorinated biphenyls;
POPs – persistent organic pollutants;
IARC - International Agency for Research on Cancer;
US EPA - U.S. Environmental Protection Agency;
BMI - Body Mass Index;
WHO – World Health Organization,
ATSDR - Agency for Toxic Substances and Disease Registry

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