Chapter 6

The diet type: vegan or traditional European (non-excluding meat) affects the content of heavy metals, dioxins and polychlorinated biphenyls in human milk

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Abstract

Introduction: Environmental pollution with heavy metals, dioxins and PCBs is a serious ecological and health problem, as they enter our bodies with the food we consume. Metals and dioxins can bioaccumulate and biomagnify, thus theoretically lower levels of toxins in the body should characterize individuals using a vegan diet that excludes products of animal origin.

Methods: The contents of heavy metals: arsenic (As), barium (Ba), chromium (Cr), zinc (Zn), cadmium (Cd), cobalt (Co), copper (Cu), nickel (Ni), lead (Pb) and mercury (Hg) as well as dioxins and polychlorinated biphenyls (PCBs) was analyzed in the breast milk of 50 women in relation to the type of diet they ate (traditional Polish or excluding meat). The concentration of metals in breast milk was determined with mass spectrometry, whereas the concentration of dioxins and PCBs was determined using gas chromatography.

Results: It was found that in some breast milk samples the content of arsenic, barium, chromium, nickel, lead and mercury exceeded admissible concentrations. Higher concentrations of copper and barium were determined in samples from women using the traditional diet, while in breast milk from women on a vegetable diet there was a higher concentration of mercury and nickel. Higher levels of heavy metals were found in breast milk samples collected in spring than in autumn. The concentration of dioxins and polychlorinated biphenyls did not exceed admissible values.

Conclusions: The ambiguous influence of the diet type on toxin concentration in human milk was observed. It cannot be stated unequivocally that vegetarian diet is a preventive factor against the accumulation of heavy metals or dioxins and PCBs in human milk.

Key words: heavy metals; dioxins; breast milk; lactation; environmental pollutants; veganism

Abbreviations

dlPCB, dioxin-like polychlorinated biphenyls; ndl-PCB, nondioxin-like polychlorinated biphenyls; LOQ, Limit of Quantification; PCDD, polychlorinated dibenzo-para-dioxins; PCDFs, polychlorinated dibenzofurans; TEQ, Toxic Equivalent; WHO-TEF – World Health Organization-Toxic Equivalency Factor

Introduction

We live in the Anthropocene age in which the natural environment has been irreversibly changed by humans and unfortunately these changes mainly encompass the destruction and continually escalating pollution of waters, soil and air. Our very own organisms are poisoned by polycyclic hydrocarbons, dioxins, polychlorinated biphenyls, heavy metals that we introduce into the surrounding environment. Pollutants along with water are taken up from the soil by plants, which then are taken up by animals, undergo bio-accumulation and bio-magnification in the subsequent links of the food chains, at the top of which are humans. As a dietary hazard for humans special consideration should be given to animal products, especially fish (also freshwater) and shellfish, as well as farm animals fed fishmeal, due to the strong accumulation of heavy metals and dioxins in both aquatic organisms [185, 186] and offal (of animal origin) [187].

However, flavonoids and antioxidants present in plants have a protective effect against damage caused by heavy metals [188, 189]. But at the same time some plants also have the ability to accumulate metals [190] and in addition, pesticides used in agriculture constitute a serious source of heavy metals, dioxins and polychlorinated biphenyls in the environment [191–199].

Anthropogenic toxins – heavy metals

Arsenic (As) is released from ores during the production of copper, lead and zinc, during coal combustion and the production of pesticides. Inorganic arsenic compounds can enter the body by the inhalation of dust, drinking contaminated water and skin contact with contaminated water or soil. This metal is accumulated in rice and the flesh of fish. Arsenic blocks sulfur residues in enzyme and it has genotoxic and carcinogenic effects [200, 201]. It is excreted from the human body in urine, where it can be detected at levels of 0.013–0.25 mg/l [202].

Barium (Ba) is used in the pharmaceutical industry, cosmetics and medicine as a contrast agent in the examination of the digestive system. Insoluble barium salts, such as barium sulphate, are considered non-toxic. Soluble and at the same time toxic barium salts include: barium chloride, barium (V) nitrate, barium (V) chlorate, barium acetate and barium carbonate, and risk of exposure occurs in the metallurgical industry and with the use of pesticides [203]. Barium toxicity results mainly from the displacement of potassium ions and precipitation of sulphate anions [204].

Chromium (Cr) at a +3 oxidation state is considered to be an essential element for health and is part of the composition of chromodulin, a tetrapeptide participating in the binding of insulin to the insulin receptor. However, chromium at a +6 oxidation state has both toxic and carcinogenic properties, easily penetrates cell membranes and can reach the cell nucleus. Chromium is one of the most commonly occurring environmental pollutants, especially waters [205, 206].

Cobalt (Co) is a micronutrient found in the body in the form of vitamin B12 (cobalamin). Inorganic cobalt is commonly found in the soil, air and food and is used in the metallurgical, ceramic and medical industries for coating prostheses and endo-prostheses. The risk of cobalt poisoning is mainly due to inhalation exposure, however excess consumption of inorganic cobalt may lead to the development of cardiomyopathy [207].

Cadmium (Cd) is found in zinc ores, is used in industry for producing battery electrodes and as a pigment. It is absorbed by inhalation or through food, especially with seafood and offal, as well as with tobacco smoke [185,191]. It accumulates in the liver and complexed by metallothionines in the kidneys. Cadmium is eliminated very slowly, its half-life in the body is about 16 years [208]. It is highly toxic, disturbing the metabolism of iron, zinc, copper, calcium, magnesium and selenium. It increases the risk of breast, uterine and prostate cancer.

Copper (Cu) is an essential microelement. It is transported by albumin and stored by hepatic ceruloplasmin. It is used as a cofactor of numerous enzymes, among others: lysyl hydroxylase, cytochrome c oxidase, superoxide dismutase, tyrosinase. Excess copper is removed in the bile. However, disturbances of homeostasis between its absorption and excretion may cause hepatitis, kidney necrosis and neurodegeneration [209].

Nickel is absorbed primarily with water. This metal often causes type IV allergy and may be neurotoxic and carcinogenic [210].

Mercury is rapidly stored and accumulated for decades in internal organs, including kidneys and the brain. Exposure to mercury can damage the nervous, excretory and immune systems [211]. Mercury occurs in the form of metallic mercury, in inorganic as well as organic compounds. It can be present in foods, specially seafood, in high concentrations.

Lead is absorbed through the digestive tract and the respiratory system, to a lesser extent through the skin. It penetrates the placenta and can be found in milk in large quantities. In the digestive tract of adults, 10% of lead present in food is absorbed, and in children this can be as high as up to 50%. Absorption of lead in the gut is reduced in the presence of calcium and phosphorus in the diet, but is increased in the presence of vitamin C. Accumulation of lead leads to damage to the nervous tissue, immune system, bone marrow, kidneys, skin and has a mutagenic and teratogenic effect [186,189,211].

Dioxins and polychlorinated biphenyls

Dioxins is a name for polychlorinated dibenzo-para-dioxins (PCDD) and polychlorinated dibenzofurans (PCDFs) (Figure 13).

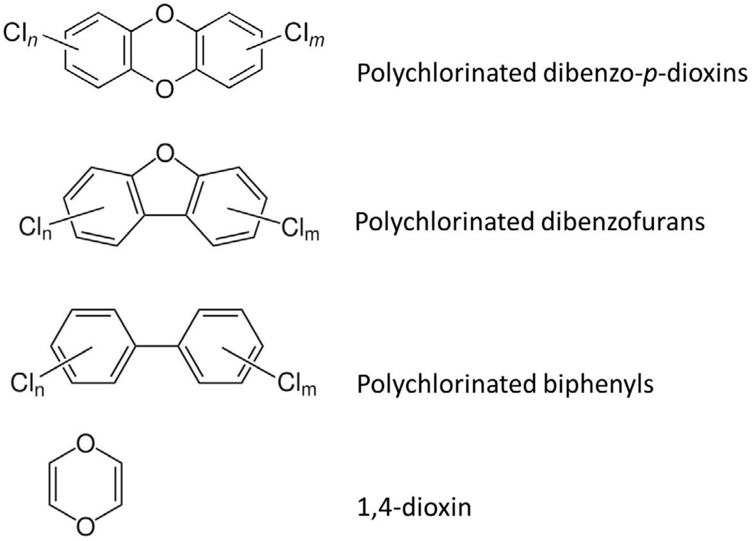

Figure 13. Dioxin structure

These compounds are formed during uncontrolled incineration of waste. Dioxins were also present in plant pesticides used in the 1980s. They are poorly water-soluble compounds, however dissolve well in fats. They have a very long half-life and bioaccumulate, especially in the adipose tissue of animals [192]. The presence of dioxins and PCBs in the body may lead to endocrine system disruption [193], delayed neurological development [194], impaired immune function, as well as mutations and carcinogenesis [195], mainly through interaction with aryl receptors. However it has also been shown that they can bind to thyroid, estrogen and androgen receptors, as well as inhibit serotonin synthesis and modify hormone levels [212,213]. Hepatic toxicity may be manifested as a result of changes in gene expression, enzyme activity, and probably as a result of damage caused by reactive oxygen species that are generated during the first phase of detoxification of these compounds on cytochrome P450 [214].

In conclusion, the negative impact of heavy metals and dioxins on the human body may include the induction of oxidative stress, intracellular transmission changes, endocrine disorders, internal organ damage, cardiovascular, respiratory, excretory, nervous and reproductive disorders, and carcinogenesis [189,191]. Toxins circulating in a woman's body during lactation can be found in human milk thereby they can also poison infant's organism.

Aim

The aim of the study was to determine whether the level of heavy metals, dioxins and polychlorinated biphenyls present in the human milk of lactating women depends on a vegan or traditional Polish diet (non-excluding meat). Due to the fact that heavy metals and dioxins may bioaccumulate and biomagnify, it was hypothesized that the lower level of tested toxins characterized people eating a vegan diet, i.e. excluding all products of animal origin, than those who consumed meat, fish, eggs and other zoonotic food products.

Methods

Tested group

The pilot study included 50 lactating women between the ages of 21 and 41. 25 women used a traditional diet including products of animal origin, while 25 women kept to a diet excluding meat, of which 9 women kept a vegetarian diet and aside from eating plant based foods included dairy products and occasional eggs and 16 women were on a purely vegetable (vegan) diet for at least three years preceding the study. The recruitment of the participants took place via an internet advertisement placed on a culinary website "Trochę Inna Cukiernia" [215] willingly followed by vegans. Participants were informed about the purpose of the study and expressed their agreement with written consent. The women participating in the study completed a survey on lifestyle, place of residence, reproductive and health history as well as a nutritional questionnaire. Breastfeeding women collected up to 3 samples of their milk between 1st and 18th week of lactation.

Nutritional questionnaire

The nutritional survey had a character of frequency questionnaire to be fulfilled by women for the four weeks before the sampling. The survey contained 176 food products with possibility to insert additional products by the participants. The number of total different food products consumed during a month before sampling. The frequency of their consumption was analyzed from very frequent: several times a day; once a day; several times a week; once a week; several times a month; once a month as very rare.

Heavy metals concentration in human milk

The 10–50 ml milk samples collected were stored at -80°C until assays analysis. The study of heavy metal content in the human milk was carried out at the Accredited Hydrogeochemical Laboratory at the AGH University of Science and Technology in Krakow using inductively coupled plasma mass spectrometry (ICP-MS) method on an ELAN 6100 spectrometer (Perkin Elmer). The content of arsenic, barium, chromium, zinc, cadmium, cobalt, copper, nickel, lead and mercury were analyzed in accordance with PN-EN ISO 17294-2: 2016-11.

Dioxins and polychlorinated biphenyls in human milk

The examination of the content of dioxins and polychlorinated biphenyls (PCBs) in human milk was carried out on 1 liter of collected samples, which was provided by four participants from Krakow and its surrounding settlements. The samples were stored at -20°C until analysis. Two of the women who provided samples for this study used a vegan diet for a minimum of three years, and two used a traditional diet containing dairy, eggs and meat. The analysis was carried out in the Accredited Trace Analysis Laboratory at the Krakow University of Technology using gas chromatography and tandem mass spectrometry GC-MS/MS according to EU regulation 709/2014 procedure P/01 issue 03 of 11/03/2010 (accreditation certificate AB 749). The average fat content of human milk was taken as 3%.

In the study the listed congeners of dioxins were analyzed: 2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TeCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF. The uncertainty of the determination of congener was estimated at 26%. Dioxin-like polychlorinated biphenyls (dlPCB) congeners were analyzed: PCB77, PCB126, PCB169, PCB81, PCB105, PCB114, PCB118, PCB123, PCB156, PCB157, PCB167 and PCB189. The uncertainty of the determination of congener was estimated at 22%. The content of PCB congeners with properties nondioxin-like polychlorinated biphenyls (ndl-PCB) were tested: PCB28, PCB52, PCB101, PCB 138, PCB153, PCB180 with The uncertainty of the determination of congeners estimated at 22%. The results were presented in accordance with the Commission Regulation (EU) No. 1259/2011 dated 02.12.2011 and as the upper limit, toxic equivalents (TEQ) were adopted as a unit.

Statistical data analysis

Statistical analysis of the collected data was carried out using the Statistica 10 softwear (StatSoft Polska). Analysis of variance was carried out using the ANOVA under the condition of positive homogeneity of variance in Levene's test and supported by post-hoc Tukey's analysis. The strength of the relationship between the variables was evaluated using the general *F* test and the Fisher procedure. To establish the existence of a correlation between the measured parameters, linear regression was used. The strength of the relationship between the variables was assessed by calculating Pearson's correlation coefficients. The results of tests where the probability of type I error was less than 0.05 were considered as statistically significant.

Results

After preliminary statistical analyses, where no significant differences between the vegan and vegetarian group were found, it was decided to combine the result pool for diets excluding meat: vegan and vegetarian. This allowed to equalize the number of analyzed groups.

Basic statistical results for the measurement of heavy metal concentrations in breast milk are presented in Table 10.

Table 10. Content of heavy metals in samples of human milk (n=124)

Metals in human milk	Mean	Minimum	Maximum	S.D.	R.A. in mammal milk	Number of samples exceeding R.A.	R.A. in water
As [mg/kg]	44.9	0.7	497.2	77.4	<100	9	<50
Ba [mg/kg]	69.1	1.7	525.3	95.3		20	<100
Cd [mg/kg]	0.6	0.1	2.7	0.7	<10	0	<5
Co [mg/kg]	14.3	1.6	70.9	14.0	0.5-7*	10	
Cr [mg/kg]	52.7	2.8	245.2	56.8		40	<50
Cu [mg/kg]	0.26	0.06	1.04	0.21	0.2-0.3*	15	
Hg [mg/kg]	4.9	0.0	74.6	9.3	<10	5	
Ni [mg/kg]	152.7	4.4	1713.7	298.3		58	<50
Pb [mg/kg]	20.8	0.1	212.9	40.6	<20	34	<10
Zn [mg/kg]	1.01	0.14	8.07	1.35	1.7-5*	5	

*S.D. – standard deviation. R.A. – recommended allowances, * typical concentration in mammal milk [201, 202]*

The concentrations of heavy metals in human milk were significantly positively correlated between each other (p <0.05). There was a correlation between the concentrations of arsenic and the concentrations of cadmium, chromium and mercury; between barium concentration and concentrations of cadmium, chromium, mercury, nickel and lead. The concentration of cadmium also correlated with chromium, mercury, nickel and lead.

It was found that there was no statistically significant correlation between the concentration of metals in breast milk and the duration of lactation nor the age of a woman in the study group nor the number of children.

In human milk from women on a traditional diet, the concentration of barium (p = 0.0134) and copper (p = 0.038; Figure 14) was significantly higher than in breast milk from vegans and vegetarians.

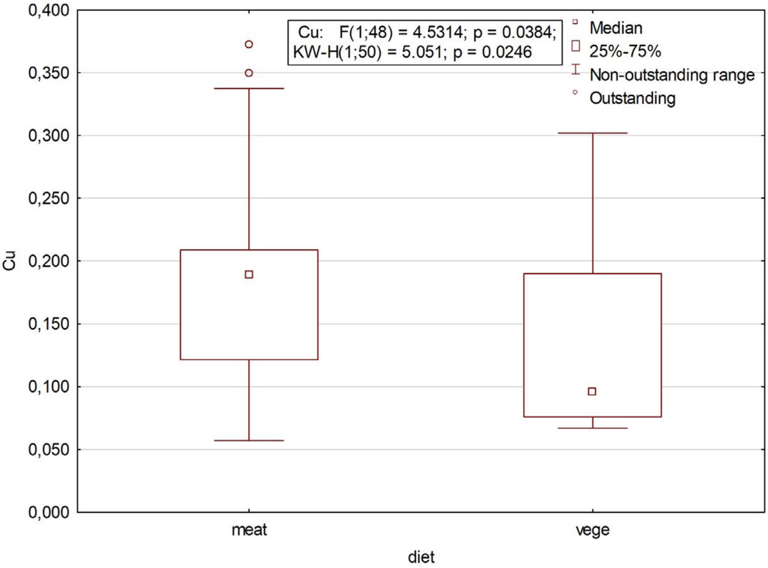

Figure 14. Relation of copper concentration in human milk samples (N=50) as measured by mass spectrometry to the women's diet: non-excluding meat (meat) or vegetarian and vegan (vege)

However, a higher concentration of mercury (p = 0.019), nickel (p = 0.035; Figure 15) and lead (p =0.036) were found in women excluding meat from their diet.

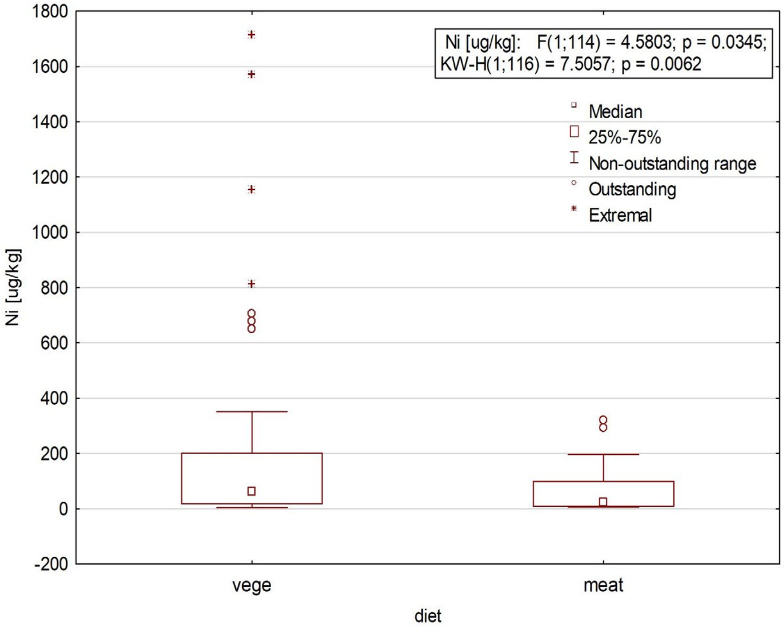

Figure 15. Correlation of nickel in human milk samples (N=50) as measured by mass spectrometry in relation to the women's diet: traditional (non-excluding meat) or vegetarian and vegan (vege)

The diet diversity distribution is shown in Figure 16. The mean number of consumed products was 85.28 (S.D. = 28.15) and median = 86.5; the minimum number of consumed food products was 35, while the maximum was 190.

The frequency of consumption of different food products was very diversified, however the high frequency of none of the analyzed products influenced the level of metals in human milk.

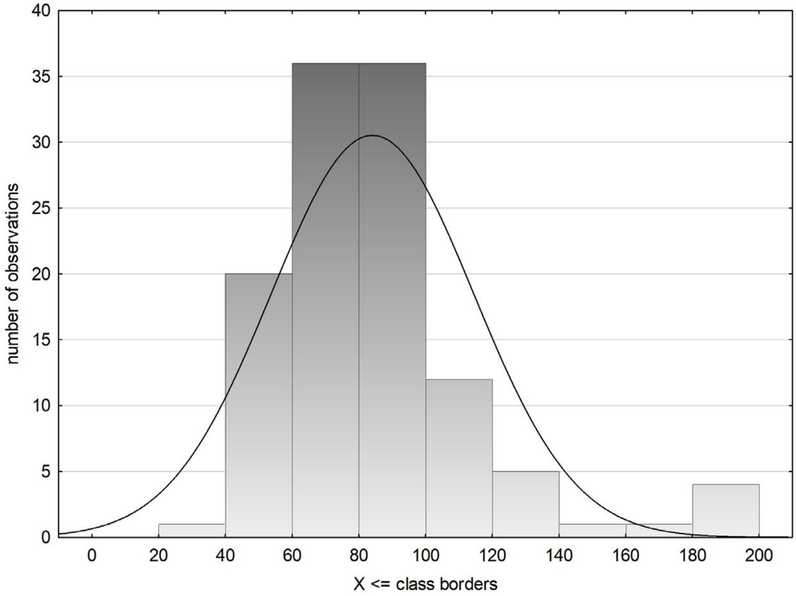

Figure 16. Food diversity among participant shown as a number of different food products consumed by the participants during one month before sampling the milk

The food diversity was independent from the diet type (vegetarian or non-excluding meat). Moreover the season of the year did not influence significantly the diversity of diet. However there was observed that the high diversity of consumed food products had an protective effect on concentration of metals in human milk: barium $p = 0.035$, copper $p = 0.003$, cobalt $p = 0.032$, mercury $p = 0.003$, nickel $p = 0.017$ and lead $p = 0.009$ (Figure 17), when the median was used as a border value for groups division in ANOVA test.

Significantly higher concentrations of heavy metals were observed in human milk samples taken between July and October than in samples collected between March to May: barium $p = 0.000$ (Figure 18); cadmium $p = 0.007$; cobalt $p = 0.000$; chromium $p = 0.001$; copper $p = 0.001$; mercury $p = 0.017$; nickel $p = 0.001$; lead $p = 0.000$.

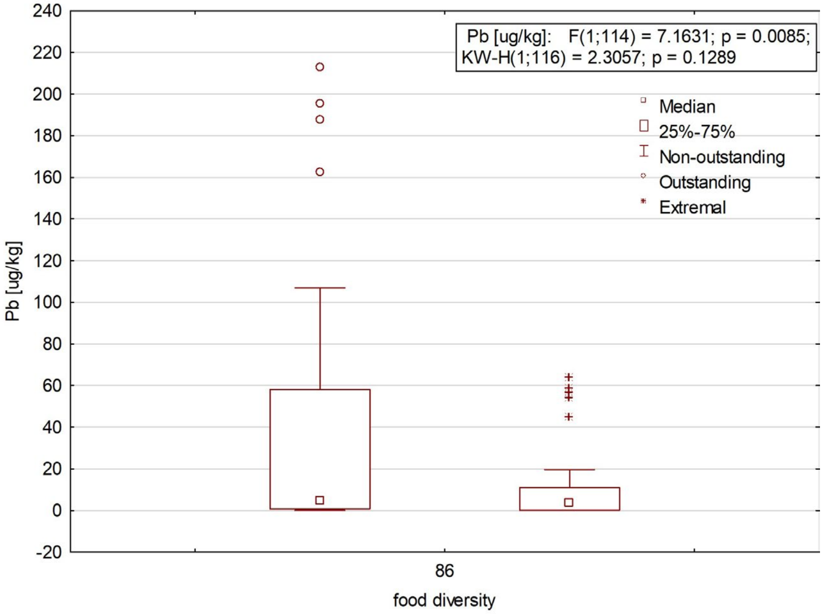

Figure 17. The concentration of lead in human milk in relation to number of food products consumed during a month before sampling. The border value for the group division was a median of number of eaten food products

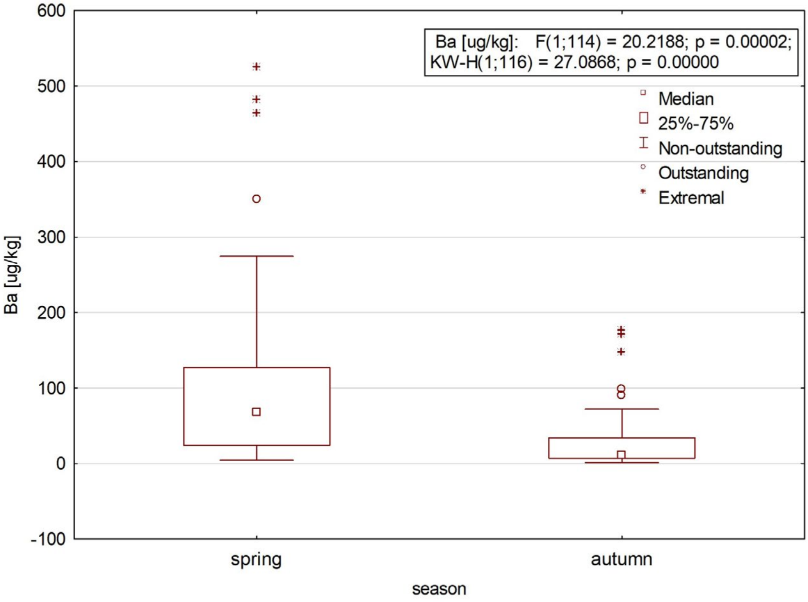

Figure 18. Correlation of barium in human milk samples (N=50) as (measured by mass spectrometry) in relation to the season when the sample was collected

The content of polychlorinated biphenyls and dioxins in human milk is shown in Table 11 and Table 12.

Table 11. Content of the sum of dioxins, dioxin-like polychlorinated biphenyls (dlPCB) and polychlorinated biphenyls with properties unlike dioxins (ndl-PCBs) in the human milk of four women from Małopolska region (Lesser Poland) expressed as TEQ equivalents [pg/g fat] ± standard deviation

Measurement	Milk 1 Traditional diet Big city	Milk 2 Traditional diet Small town	Milk 3 Vegan diet Big city	Milk 4 Vegan diet Small town
Dioxins total WHO-PCDD/F-TEQ [pg/g fat]	0.72 ± 0.19	7.0 ± 1.8	0.31 ± 0.08	0.91 ± 0.24
total dioxins and dlPCB WHO-PCDD/F-PCB-TEQ [pg/g fat]	1.30 ± 0.32	8.0 ± 2.0	0.35 ± 0.09	1.10 ± 0.27
ndl-PCB [ng/g fat]	1200 ± 2.7	35.0 ± 7.6	0.35 ± 0.08	3.10 ± 0.67

Table 12. Detailed results of dioxin and polychlorinated biphenyls content in breast milk samples from women from Małopolska expressed as TEQ equivalents [pg/g fat]

Milk sample 1, from woman on traditional diet, living in big city				
Congener	WHO-TEF	LOQ	Content	TEQ
		[pg/g fat]	[pg/g fat]	[pg/g fat]
Dioxins		0.231		0.72
2,3,7,8-TeCDD	1	0.045	n.o.	0.045
1,2,3,7,8-PeCDD	1	0.053	0.15	0.15
1,2,3,4,7,8-HxCDD	0.1	0.084	n.o.	0.0084
1,2,3,6,7,8-HxCDD	0.1	0.039	0.49	0.049
1,2,3,7,8,9-HxCDD	0.1	0.060	0.09	0.009
1,2,3,4,6,7,8-HpCDD	0.01	0.069	1	0.01
OCDD	0.0003	0.110	9.6	0.0029
2,3,7,8-TeCDF	0.1	0.069	0.45	0.045
1,2,3,7,8-PeCDF	0.03	0.094	0.5	0.015
2,3,4,7,8-PeCDF	0.3	0.110	0.83	0.25
1,2,3,4,7,8-HxCDF	0.1	0.230	0.48	0.048
1,2,3,6,7,8-HxCDF	0.1	0.150	0.39	0.039
1,2,3,7,8,9-HxCDF	0.1	0.140	n.o.	0.014
2,3,4,6,7,8-HxCDF	0.1	0.130	0.26	0.026
1,2,3,4,6,7,8-HpCDF	0.01	0.240	0.44	0.0044
1,2,3,4,7,8,9-HpCDF	0.01	0.520	0.88	0.0088
OCDF	0.0003	0.340	1.6	0.00048
dlPCB		**0.043**		**0.6**
3,3’,4,4’-TCB (PCB77)	0.0001	0.380	5.6	0.00056
3,3’,4,4’,5-PeCB (PCB126)	0.1	0.300	4.5	0.45
3,3’,4,4’,5,5’-HxCB (PCB169)	0.03	0.430	3.4	0.102
3,4,4’,5-TCB (PCB81)	0.0003	0.650	n.o.	0.0002
2,3,3’,4,4’-PeCB (PCB105)	0.00003	0.510	234	0.00702
2,3,4,4’,5-PeCB (PCB114)	0.00003	0.620	30	0.0009
2,3’,4,4’,5-PeCB (PCB118)	0.00003	0.360	1029	0.03087
2’,3,4,4’,5-PeCB (PCB123)	0.00003	0.700	13	0.00039
2,3,3’,4,4’,5-HxCB (PCB156)	0.00003	0.470	222	0.00666
2,3,3’,4,4’,5’-HxCB (PCB157)	0.00003	0.610	44	0.00132
2,3’,4,4’,5,5’-HxCB (PCB167)	0.00003	1.000	88	0.00264
2,3,3’,4,4’,5,5’-HpCB (PCB189)	0.00003	0.910	29	0.00087
		[ng/g fat]	[ng/g fat]	
ndl-PCB		**0.00523**	**12**	
PCB28		0.0024	1.9	
PCB52		0.0012	0.15	
PCB101		0.00046	0.31	
PCB138		0.00019	2.3	
PCB153		0.00058	4.4	
PCB180		0.00047	2.9	

Milk sample 2, from woman on traditional diet, living in small town				
Congener	WHO-TEF	LOQ	Content	TEQ
		[pg/g fat]	[pg/g fat]	[pg/g fat]
Dioxins		**0.772**		**7**
2,3,7,8-TeCDD	1	0.19	n.o.	0.19
1,2,3,7,8-PeCDD	1	0.18	3.7	3.7
1,2,3,4,7,8-HxCDD	0.1	0.26	n.o.	0.026
1,2,3,6,7,8-HxCDD	0.1	0.12	1.4	0.14
1,2,3,7,8,9-HxCDD	0.1	0.18	1.6	0.16
1,2,3,4,6,7,8-HpCDD	0.01	0.18	19	0.19
OCDD	0.0003	0.21	81	0.0243
2,3,7,8-TeCDF	0.1	0.34	0.63	0.063
1,2,3,7,8-PeCDF	0.03	0.28	0.4	0.012
2,3,4,7,8-PeCDF	0.3	0.39	3.9	1.17
1,2,3,4,7,8-HxCDF	0.1	0.59	9.2	0.92
1,2,3,6,7,8-HxCDF	0.1	0.34	1.8	0.18
1,2,3,7,8,9-HxCDF	0.1	0.36	n.o.	0.014
2,3,4,6,7,8-HxCDF	0.1	0.40	1.1	0.11
1,2,3,4,6,7,8-HpCDF	0.01	0.49	n.o.	0.0049
1,2,3,4,7,8,9-HpCDF	0.01	0.87	1.9	0.019
OCDF	0.0003	0.64	2.7	0.00081
dlPCB		**0.104**		**1**
3,3’,4,4’-TCB (PCB77)	0.0001	1.40	11	0.0011
3,3’,4,4’,5-PeCB (PCB126)	0.1	0.86	6.8	0.68
3,3’,4,4’,5,5’-HxCB (PCB169)	0.03	0.60	6.9	0.207
3,4,4’,5-TCB (PCB81)	0.0003	2.30	n.o.	0.00069
2,3,3’,4,4’-PeCB (PCB105)	0.00003	0.83	499	0.015
2,3,4,4’,5-PeCB (PCB114)	0.00003	0.81	74	0.0022
2,3’,4,4’,5-PeCB (PCB118)	0.00003	48.00	2403	0.072
2’,3,4,4’,5-PeCB (PCB123)	0.00003	1.10	15	0.00045
2,3,3’,4,4’,5-HxCB (PCB156)	0.00003	0.58	731	0.022
2,3,3’,4,4’,5’-HxCB (PCB157)	0.00003	0.75	142	0.004
2,3’,4,4’,5,5’-HxCB (PCB167)	0.00003	1.00	240	0.0072
2,3,3’,4,4’,5,5’-HpCB (PCB189)	0.00003	0.96	94	0.00282
		[ng/g fat]	[ng/g fat]	
ndl-PCB		**0.0133**	**35**	
PCB28		0.0096	1.2	
PCB52		0.0016	0.36	
PCB101		0.00067	0.98	
PCB138		0.00026	8	
PCB153		0.00068	14	
PCB180		0.00051	10	

Milk sample 3, from woman on vegan diet, living in big city				
Congener	WHO-TEF	LOQ	Content	TEQ
		[pg/g fat]	[pg/g fat]	[pg/g fat]
Dioxins		**0.274**		**0.31**
2,3,7,8-TeCDD	1	0.062	n.o.	0.062
1,2,3,7,8-PeCDD	1	0.059	n.o.	0.059
1,2,3,4,7,8-HxCDD	0.1	0.100	n.o.	0.01
1,2,3,6,7,8-HxCDD	0.1	0.040	n.o.	0.004
1,2,3,7,8,9-HxCDD	0.1	0.066	n.o.	0.0066
1,2,3,4,6,7,8-HpCDD	0.01	0.064	0.74	0.0074
OCDD	0.0003	0.079	6.7	0.0020
2,3,7,8-TeCDF	0.1	0.11	0.26	0.026
1,2,3,7,8-PeCDF	0.03	0.11	n.o.	0.0033
2,3,4,7,8-PeCDF	0.3	0.14	0.2	0.060
1,2,3,4,7,8-HxCDF	0.1	0.25	n.o.	0.025
1,2,3,6,7,8-HxCDF	0.1	0.16	n.o.	0.016
1,2,3,7,8,9-HxCDF	0.1	0.15	n.o.	0.015
2,3,4,6,7,8-HxCDF	0.1	0.14	n.o.	0.014
1,2,3,4,6,7,8-HpCDF	0.01	0.24	0.27	0.0027
1,2,3,4,7,8,9-HpCDF	0.01	0.47	0.54	0.0054
OCDF	0.0003	0.24	n.o.	0.000072
dlPCB		**0.039**		**0.04**
3,3’,4,4’-TCB (PCB77)	0.0001	0.59	4.6	0.00046
3,3’,4,4’,5-PeCB (PCB126)	0.1	0.32	n.o.	0.032
3,3’,4,4’,5,5’-HxCB (PCB169)	0.03	0.21	n.o.	0.0063
3,4,4’,5-TCB (PCB81)	0.0003	0.90	n.o.	0.00027
2,3,3’,4,4’-PeCB (PCB105)	0.00003	0.32	7.5	0.000225
2,3,4,4’,5-PeCB (PCB114)	0.00003	0.45	1.4	0.000042
2,3’,4,4’,5-PeCB (PCB118)	0.00003	0.23	19	0.00057
2’,3,4,4’,5-PeCB (PCB123)	0.00003	0.49	1.9	0.000057
2,3,3’,4,4’,5-HxCB (PCB156)	0.00003	0.24	3.4	0.000102
2,3,3’,4,4’,5’-HxCB (PCB157)	0.00003	0.29	0.76	0.0000228
2,3’,4,4’,5,5’-HxCB (PCB167)	0.00003	0.49	1.2	0.000036
2,3,3’,4,4’,5,5’-HpCB (PCB189)	0.00003	0.37	n.o.	0.000011
		[ng/g fat]	[ng/g fat]	
ndl-PCB		**0.0015**	**0.35**	
PCB28		0.00038	0.14	
PCB52		0.00033	0.43	
PCB101		0.00025	0.029	
PCB138		0.000086	0.034	
PCB153		0.00024	0.075	
PCB180		0.00021	0.025	

Milk sample 4, from woman on vegan diet, living in small town				
Congener	WHO-TEF	LOQ [pg/g fat]	Content [pg/g fat]	TEQ [pg/g fat]
Dioxins		**0.772**		**0.91**
2,3,7,8-TeCDD	1	0.10	n.o.	0.1
1,2,3,7,8-PeCDD	1	0.09	0.17	0.17
1,2,3,4,7,8-HxCDD	0.1	0.16	n.o.	0.016
1,2,3,6,7,8-HxCDD	0.1	0.08	0.67	0.067
1,2,3,7,8,9-HxCDD	0.1	0.12	n.o.	0.012
1,2,3,4,6,7,8-HpCDD	0.01	0.12	6.2	0.062
OCDD	0.0003	0.13	33	0.0099
2,3,7,8-TeCDF	0.1	0.14	1.4	0.14
1,2,3,7,8-PeCDF	0.03	0.16	0.3	0.009
2,3,4,7,8-PeCDF	0.3	0.22	0.26	0.078
1,2,3,4,7,8-HxCDF	0.1	0.58	1.1	0.11
1,2,3,6,7,8-HxCDF	0.1	0.36	0.56	0.056
1,2,3,7,8,9-HxCDF	0.1	0.24	n.o.	0.024
2,3,4,6,7,8-HxCDF	0.1	0.25	0.28	0.028
1,2,3,4,6,7,8-HpCDF	0.01	0.42	n.o.	0.0042
1,2,3,4,7,8,9-HpCDF	0.01	1.60	2.4	0.024
OCDF	0.0003	0.39	1.5	0.00045
dlPCB		**0.128**		**0.14**
3,3’,4,4’-TCB (PCB77)	0.0001	1.00	26	0.0026
3,3’,4,4’,5-PeCB (PCB126)	0.1	0.97	n.o.	0.1
3,3’,4,4’,5,5’-HxCB (PCB169)	0.03	1.00	n.o.	0.03
3,4,4’,5-TCB (PCB81)	0.0003	1.70	n.o.	0.0051
2,3,3’,4,4’-PeCB (PCB105)	0.00003	1.50	53	0.0016
2,3,4,4’,5-PeCB (PCB114)	0.00003	1.80	5.1	0.00015
2,3’,4,4’,5-PeCB (PCB118)	0.00003	1.10	201	0.006
2’,3,4,4’,5-PeCB (PCB123)	0.00003	2.70	7.3	0.000219
2,3,3’,4,4’,5-HxCB (PCB156)	0.00003	1.20	10	0.000
2,3,3’,4,4’,5’-HxCB (PCB157)	0.00003	1.40	1.7	0.000
2,3’,4,4’,5,5’-HxCB (PCB167)	0.00003	3.80	5.5	0.000165
2,3,3’,4,4’,5,5’-HpCB (PCB189)	0.00003	4.80	n.o.	0.00014
		[ng/g fat]	[ng/g fat]	
ndl-PCB		**0.0119**	**3.1**	
PCB28		0.0025	0.89	
PCB52		0.0035	0.54	
PCB101		0.0014	0.97	
PCB138		0.00031	0.2	
PCB153		0.0021	0.41	
PCB180		0.0021	0.056	

dlPCB, dioxin-like polychlorinated biphenyls; ndl-PCB, nondioxin-like polychlorinated biphenyls; Te – tetra; Pe – penta; Hx – heksa; Hp – hepta; LOQ, Limit of Quantification; TEQ, Toxic Equivalent; WHO-TEF – World Health Organization- Toxic Equivalency Factor

Discussion

Concentration of heavy metals in human milk

Due to the lack of established permissible standards for the concentration of heavy metals in human breast milk, the obtained results were compared to the accepted norms of metal content in milk of farmed mammals [187,216]. In the absence of developed standards for milk, it was decided to apply the accepted standards for the content of heavy metals in drinking water, as human milk constitutes the only nourishment for the first six months of an infant's life. Both these limit values are only a reference point for a safe concentration of heavy metals in breast milk. It should be taken into account that milk from farmed animals never constitutes the sole source of food for humans, which is why acceptable standards for the content of heavy metals in milk may appear to be too high when related to those for infants, and for this reason it is legitimate to use the more stringent standards for permissible concentrations of heavy metals in drinking water. It is worth mentioning that in the study group there was no high egg intake, both among vegetarians and women on a traditional diet, and what is also important to note, women using a traditional diet did not consume large amounts of fish, which both eggs and fish are often a significant source of toxins [186,196].

Unfortunately, in nine samples of human milk tested, the concentration of arsenic exceeded multiply the acceptable concentration in milk and water. The effect of diet type, place of residence or other factors on the concentration of this compound was not confirmed in comparison to pilot study [217].

It was also found that in some women's milk the content of barium exceeded the standards acceptable for those found in drinking water and it was related to diet type (higher in vegetarians) and negatively related to food diversity (lower if the diet was rich) and the season of the year (higher in spring).

It has been demonstrated that the concentration of cadmium in breast milk samples didn't exceed acceptable limits for this metal in either milk or water. The diet type used by the sample donor didn't influence the cadmium content however the higher concentrations were found in spring.

There are no set standards for cobalt in food products. It is worth mentioning that cobalt can occur not only as unfavorable inorganic compounds but also appears in the vitamin B12 as an ion coordinated in corrin ring. The analytical methods used in the study did not allow distinguishing the form in which cobalt is excreted in milk. It can only be concluded that in some samples the cobalt concentration strongly exceeded the amount of cobalt typically found in human milk as the vitamin B12 [218]. The higher concentrations of cobalt were observed in spring season.

The concentration of chromium in products for consumption should not exceed 0.05–0.1 mg/kg, unfortunately this value was exceeded in forty breastmilk samples. A statistically higher concentration of chromium was found in milk sampled in spring.

The higher concentration of copper was found in samples collected in spring than in autumn. The dangerous levels of copper (2–3 mg per day) were determined in fifteen of the analyzed milk samples. The samples collected in autumn and from women with diversified diet were characterized by lower levels of that metal.

It has been found that in more than 1/5 of all samples the concentration of lead exceeded the acceptable standards for milk and water. There was no relationship between the concentration of lead and the season of the year however higher concentrations of lead were found in milk of women using vegetarian diet and lower if the diet was rich.

Statistically higher concentrations of mercury was observed in women using a plant based diet, although theoretically a reverse dependence was expected due to the fact that its source is higher in animal products. However the diet diversity was a protective factor against high concentration of mercury in milk.

Unfortunately, in more than 1/3 of the analyzed milk samples, the nickel concentration exceeded the limits set for drinking water. The high food diversity protected against accumulation of nickel in milk. The high level of nickel in milk was found in spring season and it was related to the diet of the women as it was higher in samples from vegetarians.

The lower concentrations of barium, copper, cobalt, mercury, nickel and lead were observed in milk samples collected by women using very diversified diet who were eating more than 86 food products during a month before sampling. Then it is observed that the diet of high food diversity may protect against accumulation of toxins.

In addition, it was found that the concentrations of eight of the ten heavy metals analyzed in human milk were significantly higher in the samples collected in spring, than in the late summer and early autumn, before the start of the heating season. This is an indication that perhaps the key to increasing the level of heavy metals in the body and milk of nursing mothers is not diet but air pollution. Car fumes, heating homes with coal-fired boilers/stoves and the use of low-quality fuel cause air standards to be repeatedly exceeded in winter, this could be a probable reason for the high contamination of human milk with anthropogenic toxins as Poland is one of the most polluted countries in European Union [219].

The number of children, the duration of lactation didn't influence the heavy metals concentration in milk. However, it should be taken into account that this study did not analyze many factors that could possibly affect the concentration of heavy metals in milk, such as: the release of toxic accretions from the women's tissues, for example due to hormonal changes or changes in body weight.

Concentration of dioxin and polychlorinated biphenyls

Due to the analysis of the contents of dioxin and PCB was performed in a very small number of samples of the pilot study [217], the results of these tests cannot be subjected to statistical analysis and should be treated only as a report. The obtained values of dioxins could be related to values found in animal milk as well as in human milk from women in other populations.

According to the WHO recommendations, the maximum permissible daily allowance of PCDD dioxins is 1 pg-TEQ/kg body weight.

According to published data, female breast milk contains 25–40 pg-TEQ/g fat, while in animal milk this content is at the level of 0.1–6 pg-TEQ/g fat [196]. Therefore, assuming that an infant weighing approx. 5–8 kg, fed exclusively on human milk, consumes about 150 ml of milk with an average fat content of about 3%, it is probable that daily will receive up to 50 times the dose of dioxins than an adult with a standard diet. The results obtained in this study indicate a significantly lower content of dioxins and dioxin-like polychlorinated biphenyls (dlPCB) in the studied milk of women living in Poland than in previously published data [196]. The content of dioxins in human milk in the Netherlands [197] ranges between 4.3–32 pg-TEQ/g fat and 0.6–8.1 pg-TEQ/g fat for dioxin-like PCBs. In contrast, in Germany, the presence of dioxins in concentrations of 1.8–34.7 pg-TEQ/g fat and dlPCB 1.2–50.1 pg-TEQ/g fat [212] was found in breast milk. In China, concentrations of dioxins in human milk were found in concentrations of 7.4–23.6 pg-TEQ/g fat and dlPCB 0.9–7.9 pg-TEQ/g fat [198], while in Vietnam 1.5–14, 2 pg-TEQ/g fat [199]. Thus, the measured concentrations do not differ from the values reported in other studies and most importantly do not exceed the WHO recommended daily dose for an infant, which supports the thesis that human milk is the best solution for newborn nutrition [220], even though it sometimes has higher dioxin concentrations than in milk substitute preparations [196,221].

Conclusions

The ambiguous influence of the diet type on toxin concentration in human milk was observed. It cannot be stated unequivocally that vegetarian diet is a preventive or risk factor on the concentration of heavy metals or dioxins and PCBs in human milk. Data analysis in order to determine which environmental factors affect the content of dioxins in milk requires further testing on a larger number of samples. The issue worth of in-depth analysis seems to be the impact of the level of air pollution on the concentration of anthropogenic toxins in the milk of nursing mothers.

Women using a traditional European diet had higher concentrations of copper and barium in their milk, while samples from women excluding meat from their diet were characterized by a higher concentration of mercury, nickel and lead. However the diet diversity was found as a strong protective factor against accumulation of heavy metals in milk which is an important voice in underlining the meaning of food diversity [222–224]. Then it is to conclude that high food diversity may be more beneficial for health than exclusion of some food products.

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