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Abstract: (1) Polycyclic aromatic hydrocarbons (PAHs) are hazardous organic compounds with known carcinogenic and toxic properties. Exposure to PAHs occurs through various sources, including diet, inhalation, and skin contact, and they can transfer into breast milk following maternal exposure, potentially endangering breastfeeding infants. (2) This study aimed to determine the PAH content in breast milk samples from 50 Hungarian mothers using high-performance liquid chromatography with fluorescence detection (HPLC/FLD). The carcinogenic risk to infants was estimated using the Incremental Life Risk Calculation (IRCL) model. (3) Total PAH concentrations ranged from 0 to 78 ng/mL, with fluorene (5.3 ng/mL), phenanthrene (3.2 ng/mL), and pyrene (2.5 ng/mL) being the most abundant. PAHs were detected in 48 of the 50 samples, with phenanthrene present in 92% of samples. Dibenzo (a,h)anthracene was not detected. (4) Based on IRCL calculations, 10% of the samples were classified as safe, while 88% presented an acceptable carcinogenic risk for infants. Statistical analysis of questionnaires completed by the mothers indicated that factors such as diet, residence, and education may influence PAH levels in breast milk.

Keywords: polycyclic aromatic hydrocarbon (PAH); HPLC-FLD; breast milk; total PAHs; Incremental Life Risk Calculation (IRCL)

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed as a result of incomplete combustion of organic materials such as coal, oil, gas, wood, tobacco, charbroiled meats, garbage, or other organic materials [1–3]. They are released from natural and mainly anthropogenic sources [4].

PAHs can mean health concerns due to their probable carcinogenic, mutagenic and teratogenic effects in humans [5,6]. The degree of toxicity, carcinogenicity and mutagenicity depends on the type of PAH. The heavy PAHs (containing 4-6 rings) are more toxic and stable compared to light ones (2-3 ring) [7] (Table 1). The U.S. Environmental Protection Agency (US EPA 1993, Risk and International Cancer Agency for Research of Cancer [6,8] classified PAHs according to their carcinogenicity shown in Table 1. In addition, some PAHs classified as no-carcinogenic compounds have shown effects on the immune system and endocrine regulation [9].

Table 1. Classification of 13 EPA PAHs according to molecular weight and carcinogenicity.

Serial number	Name of the PAH	Abbreviation	MW	US EPA	IARC	TEF
1	Acenaphtylene	Acy	l	D	3	0.001
2	Fluorene	Flu	l	N/A	3	0.001
3	Phenantrene	Phen	l	D	3	0.001
4	Anthracene	Ant	l	D	3	0.01
5	Pyrene	Pyr	h	D	3	0.001
6	Benzo(a)anthracene	BaA	h	B2	2B	0.1
7	Chrysene	Chry	h	B2	2B	0.01
8	Benzo(b)fluoranthene	BbF	h	B2	2B	0.1
9	Benzo(k)fluoranthene	BkF	h	B2	2B	0.1
10	Benzo(a)pyrene	BaP	h	B2	1	1
11	Dibenzo(a.h)anthracene	DB	h	B2	2A	1
12	Benzo (g,h,i) perylene	BP	h	D	3	0.01
13	Indeno(1.2.3-c.d)pyrene	IndP	h	B2	2B	0.1

MW: Classification according to molecular weight: l: low MW (2-3 rings), h: high MW (4-6 rings); US EPA: US Environmental Protection Agency classification: Group B2 are 'possibly carcinogenic to humans' while Group D are 'unclassifiable as to carcinogenicity' NA-Not available; IARC: UN WHO International Agency for Research on Cancer classification: 1: carcinogenic, 2A probable carcinogenic, 2B: possible carcinogenic 3 not classifiable as to its carcinogenicity to humans; TEF: (Toxic Equivalency Factor) values Toxic equivalency factors/quotients (TEF/TEQs) express the toxicity of complex mixtures. For PAHs, TEF values are available for assessing their carcinogenic potential and are expressed as benzo(*a*)pyrene equivalents [10].

Human exposure of PAHs may occur via inhalation, dermal contact, or by the ingestion of food contaminated with these compounds [11,12]. Benzo(a)pyrene is generally considered the most toxic and carcinogenic PAH. The Contam panel [8] considers PAH4 (sum of benzo(*a*)anthracene (BaA), chrysene (CHr), benzo(*b*)fluoranthene (BbF), and Benzo(*a*)pyrene (BaP)) best suitable indicator for occurrence of PAHs in food. The European Commission recognizes the maximum permitted levels (EU limits) of PAHs in oils and fats as 2 µg/kg for BaP and 10 µg/kg for PAH4 [13]. The human body metabolizes and eliminates PAHs [14,15] however, the continuous exposure to PAHs in the diet and atmospheric contamination may provide a pseudo persistence [16,17].

These compounds can accumulate in lipophilic regions such as adipose tissue, liver and kidney. PAHs   may be excreted in breast milk because of their apolar character [18]. Breast milk is further susceptible for accumulating PAHs as it contains lipophilic tissue [19]. Contamination depends on environmental factors, source of exposition, stage of lactation, medical state, rearing system, and lifestyle [20,21].

There is evidence that chronic exposure in motherhood and in early childhood can have long-term consequences including birth defects, premature birth, low birth weight,malformations [22], reduced growth and development [23], asthma [24], and lower intelligence quotient (IQ) [22].

PAHs-contaminated breastmilk has been reported in numerous studies all over the world including Europe, Asia, America, and Africa: Europe: Italy [14,20,25] Portugal [26] Czech republic [27], Asia: Turkey [28], China [29], Hong Kong [30] India [31], America: [16,32,33] and Africa: Egypt [19], Ghana [34], Uganda [35].

The aim of this study is to assess the levels of PAHs in the breast milk of healthy Hungarian women and to perform a carcinogenic risk assessment to determine whether levels of PAHs in breast milk pose any risk to breastfed infants. Our aim is to further reveal factors which can have effect on the PAH concentrations in breast milk. To the best of our knowledge, this is the first study about monitoring the concentration of PAHs in breast milk of Hungarian mothers.

2. Results

Table 2. Overview of the incidence, mean, minimum, and maximum levels of PAH12 in breast milk of Hungarian nursing mothers.

PAH	Number of positive samples	Mean	Std. Deviation	Minimum detected concentration (ng/mL)	Maximum detected concentration (ng/mL)	Sum
Fluorene	26	5.34	10.52	0.50	46.08	266.91
Phenantrene	46	3.18	4.00	0.27	21.39	159.12
Anthracene	41	1.53	2.99	0.09	13.82	76.71
Pyrene	45	2.43	2.89	0.06	15.63	121.63
Benzo(a)anthracene	32	2.42	3.07	0.20	14.86	120.95
Chrysene	33	0.71	0.99	0.15	3.49	35.58
Benzo(b)fluoranthene	8	1.10	2.89	1.15	11.70	54.82
Benzo(k)fluoranthene	14	0.67	1.77	0.16	8.35	33.86
Benzo(a)pyrene	22	0.15	0.25	0.09	1.11	7.60
Dibenzo(a.h)anthracene	nd	nd	nd	nd	nd	nd
Benzo (g,h,i) perylene	18	0.17	0.28	0.16	1.43	8.38
Indeno(1.2.3-c.d) pyrene	18	0.15	0.27	0.00	1.43	7.74
ΣPAH (n=50)						

2.1. PAH Profile of Breast Milk Samples and Exposure Assessment

The concentration of 12 PAHs were determined in the breast milk of 50 Hungarian mothers. The sum of all individually measured components and 4 PAHs were also calculated in each sample and indicated as 4 PAHs and total PAHs, respectively.

Out of the 50 samples analyzed, 48 showed the presence of at least 2 PAHs, however in the other two samples no PAHs were detected. Phenanthrene was appeared most frequently (found in 92% of the samples). Dibenzo (a.h) anthracene was not detected in any of the analyzed samples. Fluorene was the most abundant PAH (5.3 ng/mL) followed by phenanthrene (3.2 ng/mL) and pyrene (2.5

ng/mL). 10 components were found in the sample, which contained the most different kind of PAHs. The concentration of 4 PAHs ranging from ND to 13.6 ng/ml with mean concentration of 4.4 ng/mL.

The most frequently detected PAH (Phenantrene) and the most abundant PAH (fluorene) was also a low molecular PAH compound, which may be transferred in human breast milk most rapidly and in greater amounts, because the mammary transport is inversely related to molecular weight [38].

The total PAHs in human breast milk ranged from 0 to 77.95 ng/mL with a mean level 17.87 ng/mL. Mean concentrations of individual PAHs in the breast milk samples of Hungarian women fell within the range of 0-6 ng/mL.

The analysis of PAH levels in the present study reveals that the detected concentrations are within a comparable range to those reported in previous research conducted in European countries, specifically Italy and the Czech Republic. This finding is consistent with previous publications [20,27] indicating a similar exposure pattern across these regions. The comparable PAH levels suggest that common sources and exposure pathways may exist, potentially related to similar environmental pollution, dietary habits, or lifestyle factors prevalent in these areas.

There is a significant trend observed consisting of higher mean levels of low molecular weight (LMW) PAHs compared to heavy PAHs. This trend aligns with findings of previous studies, where LMW PAHs-such as naphthalene and phenanthrene- were more prevalent in breast milk than heavier compounds like benzo(a)pyrene. The higher concentration of LMW PAHs may be attributed to their greater volatility and higher abundance in the environment, which facilitates their absorption and transfer into breast milk. Additionally, these compounds are often found in higher concentrations in urban areas due to traffic emissions and residential heating, which are common sources of LMW PAHs.

The consistency of these results across different geographic locations underscores the importance of monitoring PAH levels in human breast milk as a marker of environmental exposure. It also highlights the need for public health interventions aimed at reducing exposure to these hazardous compounds, particularly for breastfeeding infants who are among the most vulnerable populations. Future research should focus on identifying specific sources of PAHs in these regions, as well as examining the potential long-term health effects on infants exposed to these contaminants through breastfeeding. Such efforts are crucial for developing effective strategies to minimize exposure and protect public health.

2.2. Risk Assessment

ILCR values were calculated to estimate carcinogenic risk for infants. Out of the 50 samples, 5 had ILCR values less than 10^{-6}, which is considered safe. Only one of the samples had an ILCR value higher than 10^{-4}, indicating a potential carcinogenic risk. It is understandable that this milk sample showed a high ILCR value because it came from one of three mothers who smoke tobacco.

This sample was additionally characterized by the following factors: frequent consumption of meat, lack of organic products in the diet, no higher education qualifications, and living in an urban setting. All these factors may collectively indicate a higher risk level.

The remaining 44 samples had ILCR values within the range of 10^{-6} and 10^{-4}, which is generally considered an acceptable for breast feeding infants.

3.3. Questionnaires

Several factors that can influence the levels of PAHs in breast milk. These factors include the geographical location, residence, diet, smoking habits, and lifestyle of the individuals.

Various parameters provided by mothers in the questionnaires were analysed. We hypothesized that polycyclic aromatic hydrocarbon (PAH) compounds would occur in higher quantities in women with a higher BMI due to their lipophilic properties. However, we failed to confirm this outcome. Maternal BMI before and after pregnancy had no correlation with the total PAH concentration (p_{before}=0.262, p_{after}=0.054).

Environmental pollution may vary in different geographical areas, contributing to higher concentrations of PAHs in the air, water, soil, and food sources. Fortunately, only three mothers smoked during breastfeeding, which made statistical comparisons and conclusions unfeasible. However, the average PAH concentration was still higher in smokers compared to non-smokers, based on the data from these three mothers (smoker: 18.00±17.84 and non-smoker: 17.10±11.65).

Industrial activities, vehicle emissions, and the proximity of contaminated areas can also increase the presence of PAHs in the living environment. Individuals living in areas with higher pollution levels (such as in the capital or major cities, near busy roads or factories) may face an increased risk of exposure to PAHs, which can later affect the PAH levels in breast milk. Thus, in our analysis, we examined whether there was a significant difference in the total PAHs quantity in the breast milk of mothers living in two different areas categorized as village/municipality and town/capital. Our results confirmed that samples from mothers, living in urban environments, contained significantly more PAHs. The box plot illustrates that outstandingly high levels of PAHs are also present in mothers living in urban environments ($p<0.001$) (Figure 1).
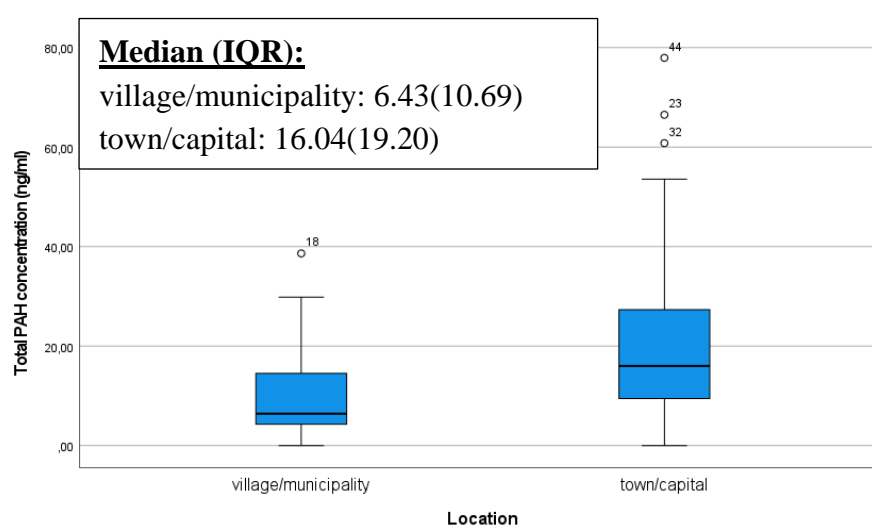
Figure 1. Comparison of total PAH concentration in breast milk samples, based on location as village/municipality or town/capital.

Our results also confirmed that diet can influence the levels of polycyclic aromatic hydrocarbons (PAHs) in breast milk, including meat, milk, and organic products that potentially affecting the overall PAH concentration. If someone consumes meat or milk contaminated with PAHs, these compounds can enter the bloodstream and subsequently be excreted into various body fluids, including breast milk [35]. Consequently, the PAH concentration in breast milk may increase with higher meat or milk consumption, especially if the meat is cooked at high temperatures or exposed to significant levels of smoke or charring. Based on our sample, mothers who consumed meat more frequently had higher PAH concentrations in their breast milk compared to those who consumed it less ($p=0.044$) (Figure 2).
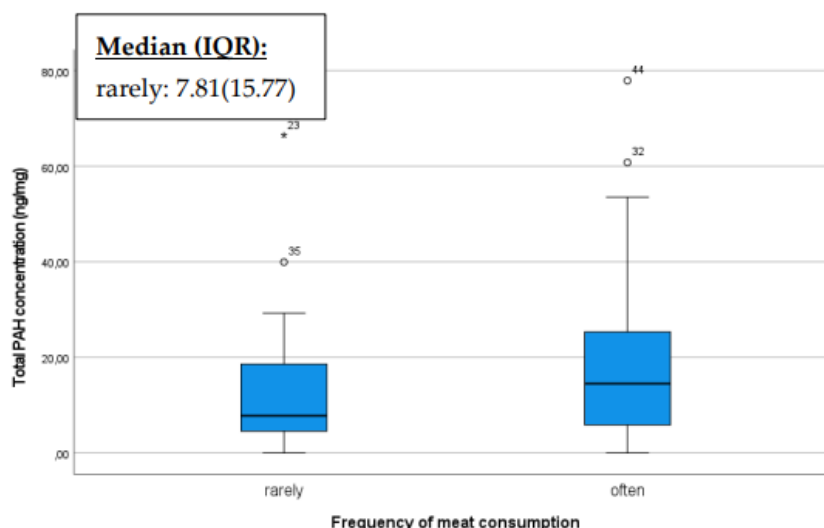
Figure 2. Comparison of total PAH concentration in breast milk samples, based on meat consumption as rarely/often.

The frequency of maternal consumption of dairy products and its influence on the PAH content of breast milk was investigated with curiosity. We obtained the result that individuals who frequently consume dairy products had a significantly higher PAH concentration ($p=0.027$) (Figure 3). PAHs are typically lipophilic (fat-soluble) compounds, and as such, they can easily accumulate in fatty tissues and animal fats. If foods such as cow milk, contain PAHs, they can be stored in the body when dissolved in fats [39].

When mothers consume dairy products, the fat content of milk provides an opportunity for the accumulation of PAHs in the body, which can then be detectable in breast milk. This is particularly noteworthy when mothers frequently consume dairy products, as the processing methods of these products, such as pasteurization or heat treatment, may potentially contribute to the formation of PAHs [31].
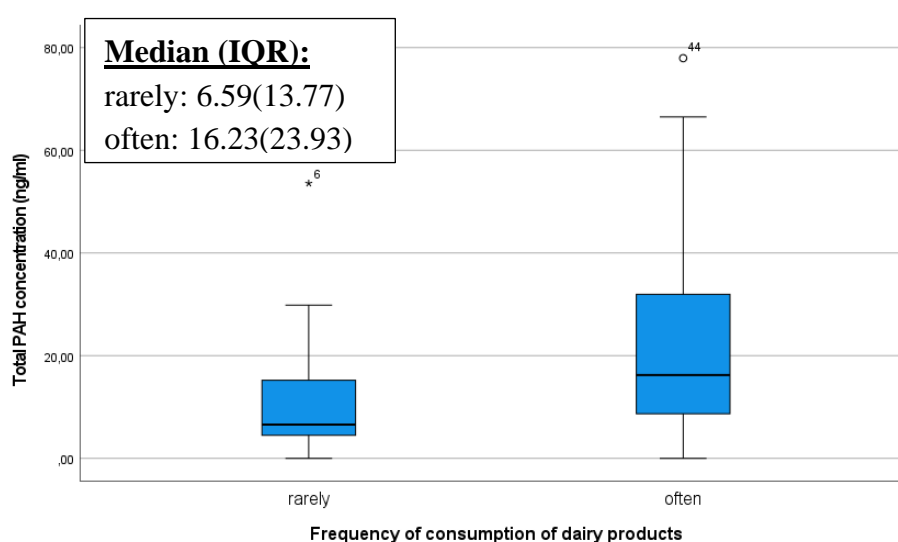
Figure 3. Comparison of total PAH concentration in breast milk, based on the frequency of consumption of dairy products of mothers.

We also asked mothers about their milk consumption habits, specifically whether they consume store-bought milk or home-produced milk. The ratio of mothers consuming home-produced milk versus store-bought milk varied significantly, with a total of ten consuming home-produced milk

and 33 consuming store-bought milk. The breast milk of mothers who consume home-produced milk had a significantly higher PAH concentration than those consuming store-bought milk (p=0.007) (Figure 4).
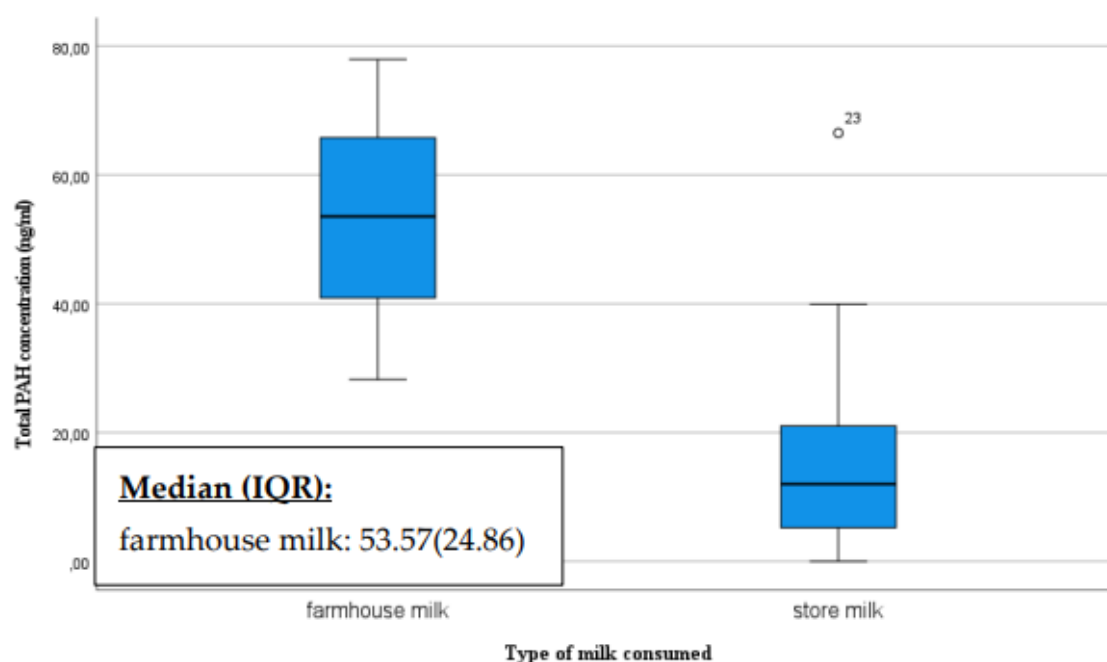
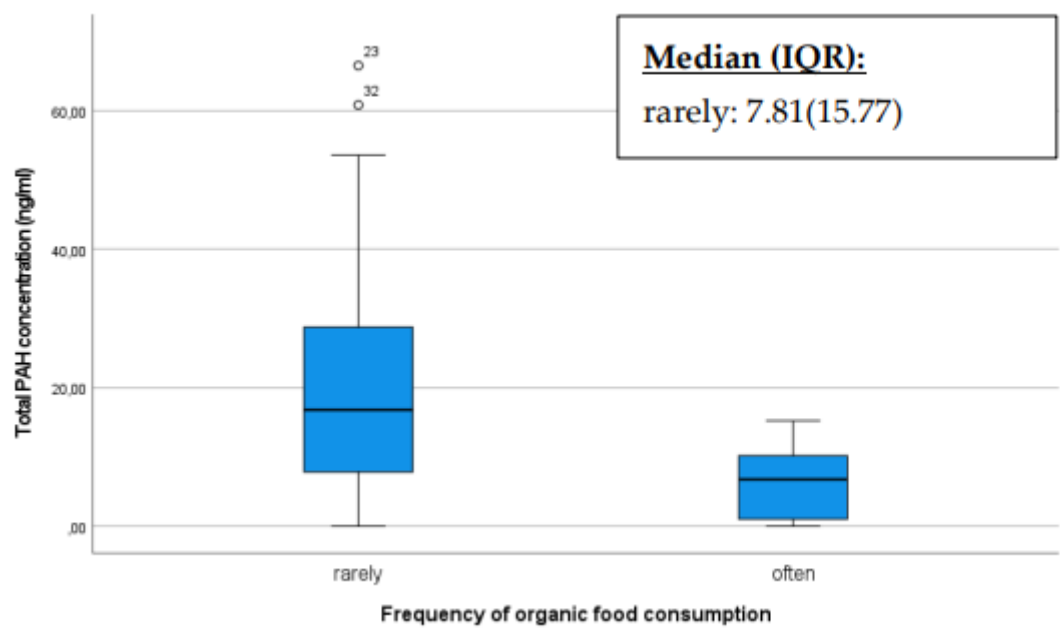**Figure 4.** Comparison of total PAH concentration in breast milk samples, based on the type of milk consumed.

The presence of PAHs in dairy products may therefore depend on the processing and preparation methods of the milk. The farmhouse milk samples may have contained PAHs levels that were higher than that detected in pasteurized milk as raw milk contained more triglycerides which may explain the higher level of PAHs [40].

Processing methods, the source of the milk, and storage conditions can vary for home-produced milk, all of which may influence the quantity of PAHs. Factors such as the diet and living conditions of the animals may also play a role. Store-bought dairy products are also processed and produced in various ways, and their PAH levels are regularly monitored to comply with standards.

Organic farming methods prioritize soil and air purity, thereby minimizing the input of chemical substances such as synthetic pesticides that may contain polycyclic aromatic hydrocarbons (PAHs) or contribute to their formation. Individuals can reduce their exposure to PAHs originating from pesticide residues by consuming organic products. The production of organic food may involve different processing methods compared to traditional foods. Producers of organic food may employ alternative processing techniques that could result in potentially lower PAH levels. In our study, we assessed how the total PAH concentration in the breast milk of mothers would differ between mothers who frequently consumed organic products compared to those that do not [41].

Our results indicate that mothers who preferentially consumed organic products had a significantly lower PAH concentration as shown in the box plot below (p<0.001). Mothers who infrequently consumed organic products exhibited more pronounced outliers with higher PAH levels (Figure 5).

Figure 5. Comparison of total PAH concentration in breast milk samples, based on the frequency of organic food consumption as rarely/often.

It is conceivable that educated or better informed mothers possess more knowledge about potential risks affecting infants, including exposure to pollutants such as polycyclic aromatic hydrocarbons (PAHs). Consequently, they are more likely to adopt healthier lifestyles and make decisions that minimize exposure to harmful substances. The box plot below also illustrates that extremely high PAH values occurred among mothers without a diploma, and their median value was significantly higher (p=0.005) (Figure 5.).
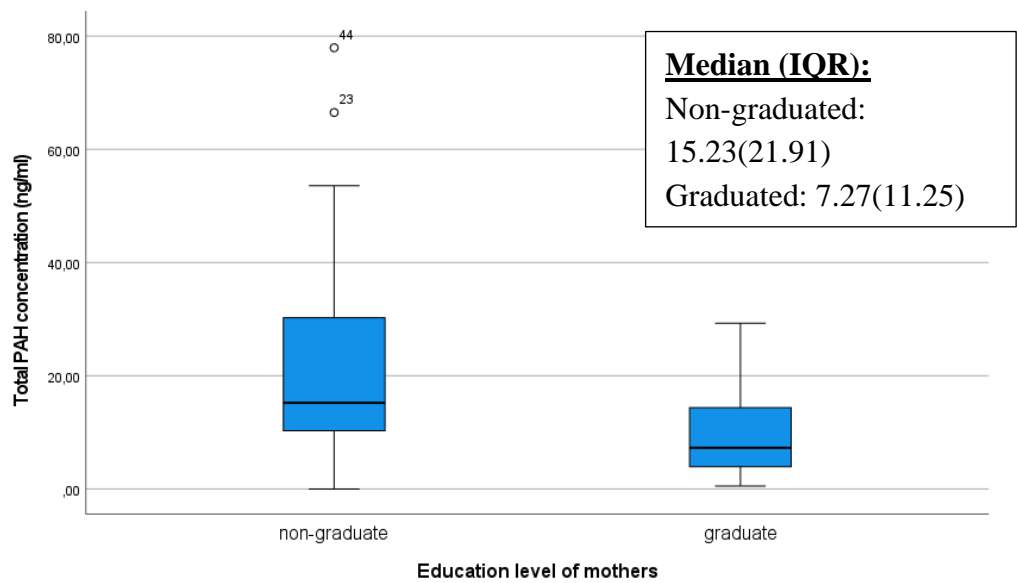
Figure 6. Comparison of the total PAH concentration in breast milk samples, based on the education level of mothers.

4. Materials and Methods

4.1. Human Subjects

A total of 50 volunteering lactating mothers, randomly selected from Hungarian population, were included in our study. The recruitment of potential donors was done with the help of the Hungarian Nursing Association (Magyar Védőnői Szolgálat). All volunteers were above the age of 18. Participating mothers signed an informed consent form and filled a questionnaire to provide information on their education, residence, dietary habits, Body Mass Index (BMI), smoking, and other relevant information which can influence the levels of PAHs in their breast milk. More details about the donors are summarized in Table 2. Permission to collect breastmilk samples in the Hungarian University of Agriculture and Life Sciences (MATE) (formerly Szent István University) was granted by the Ministry of Human Resources (decision number: 14069-6/2018/EÜIG) and the University of Pécs (Regional Research Ethics Committee, approval number: 5579) and was performed in line with the principles of the Declaration of Helsinki.

Research manuscripts reporting large datasets that are deposited in a publicly available database should specify where the data have been deposited and provide the relevant accession numbers. If the accession numbers have not yet been obtained at the time of submission, please state that they will be provided during review. They must be provided prior to publication.

Interventionary studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.

Table 2. Demographic and Health Data of Breastfeeding Mothers.

Demographic data of participating women (number of data)		Frequency (percentage)
Location (n=50)	Rural	18 (36%)
	Urban	32 (64%)
Education level (n=47)	8 classes	5 (10.6%)
	skilled worker	8 (17%)
	maturity	14 (28%)
	graduated	20 (42.6%)
Marital status (n=50)	in family	49 (98%)
	single	1 (2%)
Gravidity (n=50)	First	27 (54%)
	Second	18 (36%)
	Third	5 (10%)
Ethnicity	Caucasian White	33 (82.5%)
	Caucasian Roman	3 (7.5%)
	Asian	3 (7.5%)
	Mixed race	1 (2.5%)
BMI (before the pregnancy)	Underweight	3 (6.3%)
	Normal	28 (58.3%)
	Overweight	10 (20.8%)
	Obese I.	3 (6.3%)
	Obese II.	3 (6.3%)
	Obese III.	1 (2.1%)
BMI (after the pregnancy)	Underweight	2 (4.2%)
	Normal	21 (43.8%)
	Overweight	11 (22.9%)
	Obese I.	9 (18.8%)
	Obese II.	2 (4.2%)
	Obese III.	3 (6.3%)

The sampling of breast milk took place between 2014 and 2019. The breast milk samples were collected manually by the mothers. Approximately 10-20 ml from each participating mother were expressed and placed into clean 50 mL FALCON tubes, were frozen immediately and stored at -20 °C until the analysis. PAHs measurement were performed at the Institute of Bioanalysis at the University of Pécs.

4.2. Chemicals and Reagents

The EPA 525 PAH standard mix (500 μg/mL in dichloromethane) contained 13 PAHs acenaphthylene (**Ace)**, anthracene (**Ant)**, benzo(a)anthracene (**BaA**), benzo(a)pyrene (**BaP**), benzo(b)fluoranthene (**BbF**), benzo(g,h,i)perylene (**BP**), benzo(k)fluoranthene (**BkF**), chrysene (**Chry**), dibenzo(a,h)anthracene (**DB**), fluorene (**Flu**), indeno(1,2,3-c,d)pyrene (**IndP**) phenanthrene (**Phen**), and pyrene (**Pyr**) was purchased from Sigma Adrich, (St. Louis, MO, USA).

4.3. Sample Preparation and Chemical Analysis

The sample preparation included liquid-liquid extraction of PAHs from breast milk, followed by silica gel purification and concentration steps. Just prior to the analysis, the breast milk samples were thawed in the dark in a water bath at 37 °C for 10 minutes. An amount of 5 ml breast milk was taken to a polypropylene centrifuge tube and was mixed with 3 ml of hexane. The mixture was vortexed for 5 minutes, incubated for 30 minutes in a water bath at 37 °C and then centrifuged at 3000 rpm for 10 minutes at 4 °C. The supernatant was removed and transferred for further purification. Pasteur pipette was filled up with 2 g silica gel and was conditioned with 3 ml of 3:1 n-hexane: dichloromethane (3:1, v/v) and then washed with 3 ml of hexane, whole volume of the supernatant was put onto the silica column. The column was washed again with 3 ml of hexane and then the PAHs eluted with 3 ml of n-hexane: dichloromethane (3:1, v/v). The extract was evaporated to dryness by a gentle stream of nitrogen, then redissolved in 1 ml of acetonitrile and transferred into dark glass HPLC vials for HPLC-FLD analysis. PAHs was extracted from breast milk in duplicates. The distilled water was used as method blank and subjected to the entire analytical procedures to determine possible background interference.

All organic solvents such as hexane, cyclohexane, dichloromethane, and acetonitrile that were used for the preparation and analysis of samples were LC-MS grade (VWR International, USA).

4.4. Instrumental Analysis

HPLC-FLD separation was carried on a HPLC apparatus Shimadzu LC 20 AD model which was equipped with a binary solvent delivery system, an autosampler, a column heater, an RF-20A/RF-20Axs Fluorescence Detector (FLD), and a UV diode array detector (DAD). The chromatographic separation of PAHs was achieved on a PAH kinetex column (4.6mm x 100 mm, 3.5 μm) by Phenomenex (California, United States). The injected sample volume was 10 μl. PAHs were separated using a gradient elution program with a flow rate of 1 ml/minute. The initial mobile phase was 50% acetonitrile and 50% water for 7 min which was then gradiently changed to 100% acetonitrile over 7 min, slightly 100/0 % (8 min) and then decreased to initial phase (50-0%) over 12 min. The temperature of the column was maintained at 20°C during the analysis. The PAHs were monitored using the FLD detector. Due to the lack of fluorescent property of acenaphthylene no quantitative determination was made in case of this compound. The excitation and emission wavelengths were changed during the run to obtain the highest sensitivity for all compounds. PAHs were identified based on retention time and fluorescence spectrum, and compound quantification was performed by using external calibration method. The HPLC analysis of the samples were repeated in triplicate.

4.5. Method Validation

Different concentrations of standard calibration solutions were prepared at 9 concentration levels in acetonitrile. The calibration levels were: 0.0625, 0.125, 0.25, 0.5, 1, 5, 10, 20 and 50 ng/mL PAHs. For each level, three independent replicates were prepared in a random order. Spiked samples

(final concentrations: 5, 10 and 20 ng/mL) were prepared for recovery studies. Limit of detection (LOD) and limit of quantitation (LOQ) calculation was based on the signal to noise ratio 3:1 and 10:1 respectively. All solutions were stored in amber vials at 4 °C.

All 12 PAHs have a good linearity at 9 concentrations (0.0625, 0.125, 0.25, 0.5, 1, 5, 10, 20 and 50 ng/mL) with a regression coefficient greater than 0.9870. The LOD and LOQ of the method was ranging from 0.07-0.25 ng/mL and 0.21-0.76 ng/mL, respectively. The method performed well in the recovery of PAHs in mother milk both at low (5 ng/mL) medium (10 ng/mL) and high (20 ng/mL) concentrations. The mean recovery rates were ranging from 72.81 and 135.08 %.

4.6. Risk Assessment Analysis

4.6.1. Exposure Assessment

Toxicity Equivalent Factors (TEF) [8] have been defined as carcinogenic or probable carcinogenic PAHs. TEF values represent the relative toxicity of individual PAH compounds compared to a reference compound, which is generally applied to the most toxic, carcinogenic, and mutagenic PAH specifically BaP. The TEF value of BaP is 1. TEF values of other PAHs are shown in Table 1. PEQBaP is BaP equivalent toxicity calculated by the sum of individual PAH concentrations (C_i) multiplies with its corresponding TEF (TEF_i) as follows:

$$PEQBaP = \Sigma (C_i * TEF_i)$$

The chronic daily intake (CDI) of PAHs was calculated using the BaP equivalent concentration according to the following equation:

$$CDI \text{ (ng PEQBaP/kg bw d)} = \Sigma(C_i \times IR_i \times ED \times EF)/(BW \times AT)$$

where C_i is the PAH12 (PAH 13 standard mix without acenaphthylene calculated BaP equivalent concentration (ng PEQBaP ng/g). IR is the ingestion rate of breast milk in grams per day (700 g milk/day); EF is the exposure frequency to carcinogenic or mutagenic PAHs in days per year (350 day/year); ED is exposure duration in years (1 year); BW is the average body weight of a baby in kg (5kg) and AT is the average exposure time in days (2 years). The default values were made in consistent US EPA guidelines)

4.6.2. Risk Characterization

Estimating the infant risk for cancer related to PAH content in breast milk can involve the use of various models. We used the Incremental Lifetime Cancer Risk model (ILCR) to calculate carcinogenic risk for infants. The ILCR method is widely used in the risk assessment of PAH [36] as follows.

$$ILCR = CDI \times SF_{BaP} \times CF$$

SF_{BaP} is the carcinogenic slope factor of BaP (7.3 mg/kg), CF is the unit transformation 10^{-6} mg/ng

According to the USEPA guidelines, ILCR lower than 10^{-6} is a safe value, IRCL in the range 10^{-6} and 10^{-4} is acceptable, and IRCL higher than 10^{-4} indicate risk for infants [37].

4.7. Statistical Analysis

Statistical analysis was carried out using IBM SPSS Statistics 29 Software (IBM Corporation, Armonk, NY, USA) to find correlations between maternal dietary habits, lifestyle, and the PAH concentrations in their breast milk. A descriptive statistical analysis was performed. Data (including maternal demographic characteristics and PAH concentrations) are presented as mean ± standard deviation, or as median with interquartile range (IQR), that depended on whether the data followed a normal or non-normal distribution. The sample distribution of PAH concentrations was examined before statistical analysis using Kolgomorov-Smirnov test. The datasets followed a normal distribution in case of BaA, BbF and BkF, while in case of other PAHs, and total PAHs concentration did not follow normal distribution. Hypotheses concerning the equality of total PAHs concentration were tested using non-parametric procedures based on the Mann-Whitney U-test for comparing two different groups and Kruskal-Wallis test in case of more than two groups. Statistical significance was established as a p-value of <0.05.

5. Conclusions

The results of the study confirm the presence of PAHs in breast milk samples from Hungarian volunteering mothers. The average level of PAH concentration in breast milk is comparable to average concentrations measured in other European studies. According to the calculations made with the help of the IRCL model, most breast milk samples can be considered safe or show acceptable carcinogenic risk for infants (between 10% and 88%). Monitoring of PAHs level during lactation is recommended to follow potential changes and challenges of infant health risks. The results of this study suggest that lifestyle and dietary habits can play a significant role in regulating the concentration of PAHs in breast milk. Informing mothers about possibilities to avoid PAH affected activities (eg. smoking) and food types (eg. smoked meat) could also be important. Based on the findings, adopting a healthy lifestyle and a mindful choosing of food may contribute to a minimal quantity of PAHs in the diet, which is crucial for proper maternal and infant health. Further research should thoroughly investigate the impact of individual foods, processing methods, and other lifestyle factors on PAH concentration. Healthcare advice should emphasize the role of healthy nutrition and lifestyle in minimizing the presence of PAHs in breast milk.

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