MOTHER DIET IMPACT ON BREAST MILK QUALITY: ESTIMATION OF BREAST FED INFANTS INTAKE

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Abstract

Organochlorine pesticides, mono- and polycyclic aromatic hydrocarbon compounds were analyzed in breast milk from 169 nursing mothers from urban and rural locations of NW Transylvania in order to evaluate breast milk quality and children exposure status to selected contaminants. Range of detection for organochlorine pesticides, mono- and polycyclic aromatic hydrocarbon compounds were between 0.21–791.2, 1.44–35.1 and 0.16–140.5 ng·g^{-1} lipid weight, respectively. Maximal values were detected for organochlorine compounds, especially for DDT compound and its metabolite DDE. Correlation was obtained between regional origins of breast milk samples in case of organochlorine pesticides. Determining factors that could influence maternal milk contamination status as age, parity, lipid content were evaluated also. Evaluation of infants exposure through daily intake estimation was performed also, results showing that EDI vales ranged between 0.001–7.799, 0.001–0.651 and 0.002–0.308 µg·kg^{-1} body weight for organochlorine pesticides, mono- and polycyclic aromatic hydrocarbon compounds, respectively.

Key words: *breast milk, infants exposure, environmental contaminants, organochlorine pesticides, mono- and polycyclic aromatic hydrocarbons*

1. INTRODUCTION

It is infallible that the benefits obtained by using a wide range of products stocked from chemical industry, which are currently applied in different fields (as agriculture, industry, medicine, etc.) in order to facilitate and enhance the quality of our lifestyle, to not be reflected through a less beneficial ways (environmental contamination, health status disturbance) on our health and/or surrounding environment or biota (Mudd and Patterson, 2010; Liu et al., 2010; Cole et al., 2005; Dong et al, 2011).

Concern regarding chemical contaminants presences in any environmental media are given by their industrially advantageous physicochemical properties as increased chemical stability and resistance to degradation, properties that have led in their widespread occurrence in any kind of environmental matrix (Marti et al., 2011; Loos et al, 2010; Halsall, 2004; Jang and Townsend, 2001; Castro-Jimenez et al, 2011; Deblonde et al, 2011; Holoubek et al., 2009). Moreover, due to such properties that are often accompanied by an increased liphophilic character (Moser and McLachlan, 2001; Markwell et al., 1989; Gonzales et al, 2005), most chemical contaminants are easily bioaccumulate in fatty tissues of biological specimens (Behrooz et al, 2009; Veltman et al., 2009; Sharma et al., 2009) and biomagnificate throughout the food chain, resulting in a greater contamination level in top-predators (Harmelin-Vivien et al., 2012; Losada et al, 2009; Murai et al, 2008).

Attention regarding humans exposure to such contaminants are linked to their adverse health effects, most of them being suspected to posses carcinogenic (Farmer and Singh, 2008; Anderson, 2006; Belpomme, 2007; Boffetta, 2006), mutagenic (Coronas et al, 2009; Vargas et al, 1993) and/or teratogenic effects (Paisio et al., 2009; Sisman and Geyikoglu, 2008). Owing to these reasons, since 1980, they began to be strictly regulated or even banned from use by more and more public health, medical and environmental agencies at worldwide level.

Transylvania is a region where the agricultural and industrial activities take place in the same proportion and human settlements are placed both in rural as well urban areas. From previous studies and from the quarterly and annual reports coming from the local and national environmental agencies revealed that

in this area the presence of organochlorine compounds as pesticides and of mono- and polycyclic aromatic hydrocarbon compounds is widely commune in any kind of environmental matrix even if their levels are in most cases under the maximum permissible amount imposed by the Romanian authorities or the European Union.

Organochlorine pesticides are still widely used in large number of activities as agriculture, forestry, protection against different pests (Zhou et al., 2011). On the one hand the advantages as stability and persistence has become the principal characteristics together with their lipophilic character that highlight their possible hazard effects reported to biota. These hazards were amplified through studies that evidenced their adverse health effects (developmental, reproduction and immunological disorders; carcinogenic effects on different organs; nervous system damage; etc.) on any living beings be it laboratory animals or humans (Langer et al., 2003; Dalvie et al., 2004).

Mono- and polycyclic aromatic hydrocarbons are a chemical class that is often found in environment as a result of their formation after incomplete burning of different organic and inorganic materials as coal, oil, wood, gas, tobacco, food components, etc (Rey-Salgueiro et al., 2009). Problem of qualitative and quantitative frequencies through that they are found in any environmental matrices is amplified by the fact that a considerable number of chemical compounds belonging to this class were clearly founded by different committees and agencies as mutagenic and carcinogenic for any living beings (Commission Regulation, 2006; Codex Alimentarius Commission, 2005; European Commission, 2002; ACS, 1980;). These characteristics along with their increased liphophilic property have made that these chemicals to easily bioaccumulate in any living organisms and more over to biomagnificate through the food chain. Although in the literature are found many publications that show the presence of these compounds in different areas of concentration in a wide range of food products, publications related to their presence in human breast milk is almost inexistent.

Therefore, presence of these compounds in the surrounding environment from Transylvania is a consequence of the previous and/or actual industrial and agricultural activities.

Based on these data, the aim of this paper was to evaluate their presence in human breast milk. The importance of this study is supported by the fact that human milk is considered one of the most benefic nourishment for infants, it fulfilling all biological and health care norms (Mead, 2008; Fewtrell, 2004). Over time has been repeatedly shown that this vital food for any new born infants holds in its content a wide range of immunoprotective components or bioactive substances (Mead, 2008; Hernandez-Ledesma et al, 2007; Hamosh, 2001; Lonnerdal, 2010) which posses multiple health benefits as reduction of infection or diseases, and that cannot be substituted by any infant formula (Lawrence and Pane, 2007; Haddy and Adams, 1952).

It is well known that human breast milk is one of the most suitable biomarker for women exposure assessment to environmental contaminants, because it contains lipids originating directly from their adipose tissue (Meydan and Sezerman, 2010). Also this kind of matrix is considered to belong to non-invasively way collected biological matrices, thus its collection and analysis didn't causing any discomfort, pain or harm to the monitored subjects (Esteban and Castano, 2009). Another advantage of used of this biological matrix in ecototoxicological surveys is given by the fact that its analysis and evaluation not only provides information about studied mothers' exposure but also it provides information regarding early exposure to environmental contaminants of nurslings (Li et al, 2008; Angulo et al, 1999; Nickerson, 2006).

Although chemical contaminants presence in breast milk has started to be reported from 1951, when was evidenced for the first time the DDT presence in human bodies, moment hence more and more studies have been performed in this line (Wong et al, 2005; Bradt and Herrenkohl, 1976), in our countries no reports were published yet regarding environmental contaminants presence in mother milks.

2. MATERIALS AND METHODS

2.1. Chemicals and standard solutions

Organochlorine pesticide mix standards – EPA CLP Organochlorine pesticides mix at 2000 μg·mL^{-1} in hexane:toluene (1:1) – including: aldrin, α-BHC, β-BHC, lindane (γ-BHC), δ-BHC, α-chlordane, γ-chlordane, 4.4'-DDD, 4.4"-DDE, 4.4'-DDT, dieldrin, α-endosulfan, β-endosulfan, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide isomer B, methoxychlor, were purchase from Supelco (Bellefonte, PA, USA).

Polycyclic aromatic hydrocarbons mix standard – QTM PAH Mix at 2000 μg·mL^{-1} in methylene chloride – including: acenaphthene, acenaphthylene, anthracene, benz[a]anthracene, benzo[b]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, 2-bromonaphthalene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorine, indeno[1.2.3-cd]pyrene, naphthalene, phenanthrene, pyrene, were purchase also from Supelco (Bellefonte, PA, USA).

Monocyclic aromatic hydrocarbons as BTEX Mix at 2000 μg·mL^{-1} in methanol including benzene, ethylbenzene, toluene, o-xylene, m-xylene and p-xylene was achieved so from Supelco Bellefonte, PA, USA).

Standard working solutions were prepared in n-hexane at concentration of 100 μg·mL^{-1} and 4 μg·mL^{-1}, respectively which was used in performing of method optimization and method validation experiments.

Working solvents as n-hexane (chromasolv grade, ≥ 97.0 %) and dichloromethane (chromasolv plus grade ≥ 99.9 %) were achieved from Sigma-Aldrich (St. Louis MO, USA). Methanol (LC-MS ultra chromasolv grade, ≥ 99.9 %), anhydrous $NaSO_4$ and NaCl concentrate were purchased from Fluka (Buchs, Switzerland).

2.2. Sampling and sample preparation

2.2.1. Sampling sites and sampling strategy description

We selected for the study three counties, namely Cluj, Salaj and Bistrita Nasaud, located in the northwestern part of Transylvania. These three counties lie between Apuseni Mountains and the eastern Carpathians mountains, region known as Somes River Platform, name that comes from the main river (Somes River) that crosses these hilly areas. Activities from this region are divided proportionally between industrial and agricultural activities and human settlements are distributed about the same percentage between rural and urban settlements (table 1).

County	Area (km^2)	Population*	No. of breast milk donors	
			Rural area	Urban area
Salaj	3 864	243 157	31	21
Cluj	5 355	277 861	22	47
Bistrita-Nasaud	6 674	659 370	27	21

*According with Regional Statistics Directorate for 2011, Romania.

Table 1. General characteristics of studied counties and monitored mothers distribution through studied areas.

Totally 169 nursing mothers ranging in age from 15 to 38 years and without serious clinical complications were monitored between periods of 2008 -2010. All involved mothers were recruited randomly and managed on a voluntary basis but meeting some major criteria as: completeness of all items in the questionnaire; minimum duration of living in the same residence for five years; able to provide enough breast milk to conduct the monitoring studies (over 50 mL). From all breast milk donors

about 50 mL – 100 mL of milk samples were collected in sterile condition, usually at the hospital where the children birth took place. All amount of milk samples were collected in sterilized chemical-free brown glass bottles and sealed with Teflon screw caps. Samples were transported as soon as possible to laboratory where were frozen at -20 °C until analysis. All analysis was performed within 7 days starting from the sampling date. Before to start breast milk sample preparation for target compounds analysis, all milk samples stored in frozen conditions were brought to room temperature (21 °C) and homogenized by manual mixing for 10 minutes.

2.2.2. Lipid content determination from breast milk

Total amount of lipids from breast milk were determined gravimetrically according with methods described previously by Lopez-Espinosa et al, (2007), Ennaceur et al, (2008), Jaraczewska et al., (2006) and Kampire et al., (2011). Briefly the following two methods were used in order to compare the accuracy of obtained results: (a.) 10 mL of breast milk were placed in a centrifuge vials and centrifuged over 20 minutes at 7 500 rpm after that the upper layer composed by total fat was removed and quantified gravimetrically; (b.) 5 mL of human breast milk were used from every samples for extraction with 15 mL hexane:ethanol (2:1 v/v) for 5 minutes. This procedure was repeated three times after that the organic layer was passed through a 5 mL polypropylene cartridge filled with anhydrous $NaSO_4$. The obtained aliquot was evaporated to dryness under gentle nitrogen stream after that the lipids were measured gravimetrically (Jaraczewska et al, 2006).

2.2.3. Breast milk samples treatment for organochlorine pesticides analysis

Sample treatment in order to analysis organochlorine compounds were done through headspace-solid phase microextraction (HS-SPME) technique in automatic mode with chromatographic HS-SPME autosampler according to methods described in detail in the work done by Fernandez-Alvarez et al., (2008) and Rodriques et al., (2011) introducing only small changes as follows: 10 mL of breast milk sample were placed in 22 mL sterilized headspace glass vials with 2.8 mg NaCl (in order to achieve salting out effect) and sealed with Teflon screw caps.

Apolar polydimethylsiloxane polydimethylsiloxane (PDMS) fiber with 100 μm coating and 1 cm fiber length were used according to the target compounds that wanted to be analyzed, in order to perform the extraction process. Sample equilibration was set at 80 °C for 10 minutes under continuous stirring conditions using the autosampler oven. Extractions of target organochlorine analytes were done by exposing the SPME fibers to breast milk sample for 20 minutes. Target analytes thermal desorption was achieved over 5 minutes by inserting the exposed SPME fiber into the gas chromatographic (GC) injector port which was set at 280 °C and under splitless condition.

2.2.4. Breast milk samples treatment for mono- and polycyclic aromatic hydrocarbons analysis

Monoaromatic hydrocarbons as benzene, toluene, ethylbenzene, xylenes (m-xylene, p-xylene and o-xylene) and low molecular polycyclic aromatic hydrocarbons as naphthalene, acenapthalene and phenanthrene were extracted using HS-SPME technique with a 100 μm PDMS fiber as method described by Aguinaga et al. (2007) with small changes introduced. Shortly, a 100 μm PDMS fiber coating was inserted in the headspace over the 10 mL milk sample placed in a 22 mL headspace glass vials that before was sealed with a Teflon screw caps and equilibrated at 60 °C for 45 min time period under continuous stirring. 30 minutes adsorption onto the PDMS fiber of these target analytes was considered the optimal. These analytes thermal desorption took 5 minutes and was made by inserting the exposed fiber into the GC injector port which was set at 280 °C and under splitless condition. The remaining high molecular polycyclic aromatic hydrocarbons were also extracted using SPME technique but this time a bipolar fiber was used, namely a 65 μm PDMS-DVB fiber which in this case was directly immersed in the preheated milk samples (at 85 °C over 30 minutes time period under continuous stirring condition) for 15 minutes. After that the target analytes adsorption on the PDMS-DVB fiber was obtained, the fiber was introduced in the GC splitless injectior port set at 280 °C where the analytes thermal desorption take place during 10 minutes. The inconvenient of these extraction procedures has been given by the fact that a fiber had to be replaced after approximately 30-40 breast milk sample analysis.

2.3. Instrumental analysis of target contaminants from breast milk samples

2.3.1. Organochlorine pesticides instrumental analysis

Organochlorine compounds with their metabolites were analyzer by Trace GC Ultra gas chromatography using ^{63}Ni electron capture detection (GC-ECD, Thermo Electron Corporation, USA) and conformed also by Focus GC gas chromatography with DSQ II quadupole mass spectrometer detector (GC-MS, Thermo Electron Corporation, USA), both equipped with split/splitless injection port. Obtained GC-ECD chromatograms were interpreted using ChromCard software (Thermo Electron Corporation, USA). Chromatographic separation of monitored organochlorine compounds on GC-ECD was done using a TR-1701 capillary column (14 % Cyanopropylphenyl polysiloxane, Thermo Electron Corporation, USA) with 30 m length x 0.32 mm. I.D., x 0.25 µm film thickness. High purity of nitrogen was used as carrier and make-up gas with a 1.5 mL·min^{-1} flow. In order to achieve target organochlorine analytes separation the GC oven temperature program was set as follow: 40 °C (hold for 3 min) followed by an increase of 7 °C·min^{-1} to 165 °C, and continued growth with 15 °C·min^{-1} to 280 °C and maintained at this final temperature for 5 minutes. The total acquisition time of this program was 27.14 min. Chromatograph injection port as well electron capture detector temperature was set at 280 and 300 °C, respectively. All samples were also checked on GC-MS using a TR-5 MS capillary column with 30 m length x 0.32 mm. I.D., coated with a 0.25 µm film thickness of 5 % phenyl polysilphenylene siloxane. Selected ion monitoring mode (SIM) was used and chromatographic and mass spectrometric data were recorded and processed by the XCalibur software (Thermo Electron Corporation, USA).

2.3.2. Mono- and polycyclic aromatic hydrocarbons instrumental analysis

Monocyclic aromatic hydrocarbons (as benzene, toluene, ethylbenzene and xylene with its isomers as m-, p- and o- xylene) and the 16 target components of polycyclic aromatic hydrocarbon compounds were detected on Focus GC gas chromatography with DSQ II quadupole mass spectrometer detector (GC-MS, Thermo Electron Corporation, USA) provided with a split/splitless injection port. TR-5 MS capillary column (5 % diphenyl – 95 % dimethyl polysiloxane, Thermo Electron Corporation, USA) with 30 m length x 0.25 mm. I.D., x 0.25 µm film thickness was used for chromatographic separation of mono- and polycyclic aromatic hydrocarbon compounds. Oven temperature was set as follows: 60 °C (hold constant for 3 min) followed by a heating at 100 °C with a rate of 10 °C·min^{-1}, after which the temperature raising continued at 280 °C with a rate of 15 °C·min^{-1}. This final temperature (280 °C) was maintained constant for 10 minutes. SIM mode was used during GC-MS analysis and the groups of ions are given in table 2. Chromatographic and mass spectrometric data were recorded and processed by the XCalibur software (Thermo Electron Corporation, USA). Selected ion monitoring mode (SIM) was used and chromatographic and mass spectrometric data were recorded and processed by the XCalibur software (Thermo Electron Corporation, USA).

2.4. Quality assurance

Methods performance was evaluated for both contaminants classes. Blank milk samples were analyzed routinely at every fourth breast milk samples, samples where were not observed the evidence of chromatographic peaks of the target monitored contaminants in their chromatograms.

Limits of detection (LOD) on the target organochlorine pesticides as well mono- and polycyclic aromatic hydrocarbons were established by calculating the concentration of the target analytes that correspond to three times to the background noise level. LOD values for organochlorine pesticides vary between 0.002 – 0.012 µg·L^{-1} while for mono- and polycyclic aromatic hydrocarbons between 0.004 – 0.118 and 0.006 – 0.015 µg·L^{-1}, respectively, which correspond to 0.05 – 0.3 ng·g^{-1} milk fat, 0.1 – 2.95 ng·g^{-1} milk fat and 0.15- 0.37 ng·g^{-1} milk fat after that fat content was determined gravimetrically.

Extraction methods recovery was performed on spiked blank breast milk samples (n = 10) with the following amounts of organochlorine and mono- and polycyclic aromatic hydrocarbon compounds: 1 µg·L^{-1}, 5 µg·L^{-1} and 10 µg·L^{-1}. The spiked samples were left for equilibration for 12 hours before to perform the extraction and analysis, respectively. All samples from the recovery assays were repeated

three times. In cases of all target contaminants the recovery values ranged between 73 – 122 %. No additional corrections for recoveries were added to the final results.

2.5. Statistical interpretation of results

Statistical analysis were done using Statistica 8.5 program. Descriptive statistic parameters as mean, median and/or standard deviation were computed to characterize the distribution of organochlorine pesticides, mono- and polycyclic aromatic hydrocarbons amount in analyzed human breast milk samples.

2.6. Questionnaires

All monitored breastfeeding mothers were asked to provide information on: their age and as appropriate about the age that bore before; on their parity; height and weight during pregnancy; infants' birth weight and length; breast-feeding history, possible exposure history coming from working places and/or previous places of residence; dietary, smoking and/or drug habit. All these information were included in the questionnaires that after completion were processed and used to interpret the results obtained from the monitoring study.

2.7. Ethical conduct of research

Through this study were followed the code of ethics established by the revised version from 2008 of Helsinki Declaration from 1964 regarding ethical principles of medical researches involving humans (WMA, 2008). All monitored mothers participate voluntarily in this study and all of them were well informed about the aim of this study and potential consequences, thus all of them signed and informed consent form. Monitored mothers offers through questionnaires detailed information regarding their age, weight and height, possible exposure at workplace and/or throughout the course of their lives, living area, dietary and smoking habit, medical history record, etc.

3. RESULTS

3.1. Subjects description

A total number of 169 nursing mothers were involved in this study. In a roughly similar percentage, mothers were selected from both rural (n = 80) and urban (n = 89) areas of the northwest region of Transylvania. For this monitoring program were selected healthy mothers who had no risk with the pregnancy, although even then a total of 12 mothers had complication through delivery moment. Between monitored mothers were both primparous (n = 98) as well multiparous (n = 71). Commonly, mothers living in rural areas were younger than those from urban areas, 73.75 % from monitored mothers from rural area were under the age of 25 (≤ 25 years) while 53.93 % of mothers from urban areas were over 26 years old (≥ 26 years) when they gave birth to first child. In case of rural mothers only four were older than 26 years when they born the first child, the rest of than this age giving birth to two or more children over this age. Same behavior was observed also for number of deliveries between the two studied groups, thus in case of mothers from urban areas just 36 (40.44 %) were multiparous (n ≥ 2, but didn't exceeding the number of three deliveries). These can be motivated by differences between the interests and concepts of the two categories, habitually for mothers living in urban areas the focus is on professional and material achievement while in case of mothers from rural areas the emphasis are on family. Mothers from both studied groups were asked through questionnaires to provide rigorous information regarding their diet during pregnancy and breastfeeding period. Centralized data related to maternal diet are presented in table 2.

Frequency (no. of person; percentage %)	Dietary habits over normal weekly food consumption								
	Meat				Dietary products		Eggs	Veg*.	Fruits
	Poultry	Cow	Pig	Fish	Milk	Others			
Never	15.97	28.99	17.15	32.54	7.69	1.18	12.42	none	1.18
Low (1 time)	25.44	27.81	14.2	24.26	17.15	15.97	26.03	1.77	17.15
Moderate (2-4 times)	47.33	40.23	50.88	40.23	47.92	53.25	49.7	28.99	52.66
High (> 4)	11.24	2.95	17.75	2.95	27.21	29.58	11.83	69.23	28.99

*Veg. – vegetables

Table 2. Mothers weekly foodstuffs consumption (expressed as: number of person; %).

According with information provided by these questionnaires, revealed that mothers form rural areas have a much loaded died (in terms of meat, dairy products and egg consumed) than in case of mothers from urban areas where the consumed food were more balanced, meat products were appropriate alternated with vegetable and fruit products. Mothers' modes of diet have reflected on maternal milk quality, so for those with a rich and diverse diet the maternal milk was also richer in fat content. From the totally of 169 monitored mothers just 19 parsons were lacto-ovo-vegetarians, seven persons from rural areas and twelve from urban areas.

3.2. Organochlorine pesticides presence in human breast milk

Presences of 21 organochlorine pesticide compounds were monitored during this study. Their selection was based on both their popularity in uses and the strictness with which they were included in various international and national legislative monitoring programs, most of them being considered as priority hazardous substances. At least one of these target compounds was detected in every pooled milk samples which lead to conclude that the general population from northwestern part of Transylvania has been widely exposed over time to these chemicals. Among the 21 analyzed compounds DDT (85.79 % detection; n = 169) with its metabolite 4.4'-DDE (85.79 % detection; n = 169) were the most predominant in breast milk samples collected from all monitored areas. Briefly, the frequency of detection (% from n = 169) and the quantitative amount of these monitored pesticides in breast milk samples usually followed this order: DDT (85.79 %; range: 0.21 - 791.29 $ng \cdot g^{-1}$ lipid weight) > DDE (85.79 %; range: 2.91 – 291.31$ng \cdot g^{-1}$ lipid weight) > aldrin (56.8 %; range: 1.22 – 397.34 $ng \cdot g^{-1}$ lipid weight) > β-BCH (43.78 %; average: 0.73 – 352.4 $ng \cdot g^{-1}$ lipid weight) > heptachlor (31.95 %; range: 0.87 – 296.46 $ng \cdot g^{-1}$ lipid weight;) > endrin (37.86 %; range: 0.61 – 275.62 $ng \cdot g^{-1}$ lipid weight) > α-endosulfan (43.78 %; range: 0.31 – 216.38 $ng \cdot g^{-1}$ lipid weight) > α-chlordane (36.09; range: 0.9 – 171.82 $ng \cdot g^{-1}$ lipid weight) > lindan (48.52 %; range: 1.07 – 141.07 $ng \cdot g^{-1}$ lipid weight) where the corresponding average values from the total analyzed breast milk samples were 50.11, 47.77, 17.82, 13.32, 16.62, 13.08, 9.3, 9.18 and 11.85 $ng \cdot g^{-1}$ lipid weight, respectively. Minimal amounts were detected in case of δ-BHC (average: 4.14 $ng \cdot g^{-1}$ lipid weight; frequency: 26.62 %), β-endosulfan (average: 2.5 $ng \cdot g^{-1}$ lipid weight; frequency: 24.26 %), endosulfan sulphate (average: 4.52 $ng \cdot g^{-1}$ lipid weight; frequency: 29.58 %), endrin ketone (average: 4.95 $ng \cdot g^{-1}$ lipid weight; frequency: 29.58 %) and methoxychlor (average: 4.88 $ng \cdot g^{-1}$ lipid weight; frequency: 20.11 %). Extended data regarding frequency of detection, average amount and minimal and maximal values detected for all monitored organochlorine pesticides are given in table 1 from supplementary materials.

3.3. Mono- and polycyclic aromatic hydrocarbon presence in human breast milk

Through this study the presence of 16 polycyclic aromatic hydrocarbons and 5 monoaromatic hydrocarbons were followed on all maternal milk samples collected. From the total number of breast milk samples (n=169) in 41.42 % were detected at list one mono- or polycyclic aromatic hydrocarbon

compound, maternal milk samples from 70 breastfeeding mothers being completely free from any of these monitored compounds. In the highest percentage were detected the following mono- and polycyclic aromatic compounds: toluene (20.11 %), naphthalene (45.56 %), benz[ghi]perylene (37.27), chrysene (36.09 %), benz[b]fluoranthene (34.31 %), benz[a]anthracene (31.95 %) and dibenz[a,b]anthracene with benz[a]pyrene (33.13 %). The maximal values that were detected in case mono- and polycyclic aromatic compounds were: 35.11 $ng \cdot g^{-1}$ lipid w. (benzene), 140.52 $ng \cdot g^{-1}$ lipid w. (anthracene), 110.65 $ng \cdot g^{-1}$ lipid w. (benz[b]fluoranthene), 91.95 $ng \cdot g^{-1}$ lipid w. (phenanthrene), 91.37 $ng \cdot g^{-1}$ lipid w. (chrysene) and 88.15 $ng \cdot g^{-1}$ lipid w. (pyrene). Low values and low detection frequencies were registered in case of acenaphthene (maximal value: 30.28 $ng \cdot g^{-1}$ lipid w.; 23.66 % detection), acenaphthylene (maximal value: 21.38 $ng \cdot g^{-1}$ lipid w.; 21.3 % detection) and o-xylene (maximal value: 8.01 $ng \cdot g^{-1}$ lipid w.; 8.28 % detection). Increased average value from the total number of analyzed breast milk samples (n=169) were obtained in case of benz[ghi]perylene (8.41 $ng \cdot g^{-1}$ lipid w.), naphthalene (7.1 $ng \cdot g^{-1}$ lipid w.), benz[a]perylene (6.93 $ng \cdot g^{-1}$ lipid w.), benz[b]fluoranthene (6.77 $ng \cdot g^{-1}$ lipid w.), chrysene (6.31 $ng \cdot g^{-1}$ lipid w.) and benz[a]anthracene (6.28 $ng \cdot g^{-1}$ lipid w.).

Significantly low values were observed in case of monoaromatic hydrocarbons, their range varying between 1.44 – 35.11 $ng \cdot g^{-1}$ lipids w., they presenting also a low frequency of detection from the total monitored nursing mothers' breast milk samples (maximal detection being registered in case of toluene – 20.11 % from total samples). Full view of results regarding mono- and polycyclic aromatic hydrocarbon compounds including frequency of detection, average amount and minimal and maximal values detected are given in table 2 from supplementary materials.

4. DISCUSSIONS

4.1. Comparison of organochlorine pesticides, mono- and polycyclic aromatic hydrocarbons presence in human breast milk with literature data

Environmental contaminants as organochlorine pesticides are one of the classes of chemicals that were most intensively monitored and studied over time, they being highlighted in biological samples since 1980's. Thus, starting from the emphasizing of adverse effects on humans' health as well their pointing out in different biological matrixes, a growing number of studies have been conducted in this direction. Although at worldwide level there are numerous publications reporting qualitative and quantitative presence of pesticides in biological samples like human breast milk samples, such information has not been reported yet from our country (Romania). In table 3, we cited values reported from worldwide for the most frequently detected organochlorine pesticide residues in human breast milks. If we compare these data with our obtained values we can observed that the contamination status in our studied regions is low in case of DDT primary metabolite, DDE (ranged between: 2.91 – 291.31 $ng \cdot g^{-1}$ l. wt.), than in case of other countries from Europe where the maximum could reach values of thousands as case of Poland, Czech or Russia or including countries from other continents, as China, India, Iran or Brazil (table 3.). In case of parent compounds, namely DDT, results are different from those reported worldwide, namely in this study were detected much higher levels, with almost three times (0.21 – 791 $ng \cdot g^{-1}$ l. wt.), if we compare them with other countries from Europe (see table 3), data that implies that human exposure to DDT still occur. Problem of continuous exposure to DDT could be explained by the fact that fresh input of DDT still occur during agricultural practices. Although this is illegal under the Stockholm Convention, in Romanian legislation are not provided regulations regarding the use of any pesticides as long as obtained agricultural products are only for personal use and not for commercial purposes. In case of BHC compounds the amount detected in breast milk samples were higher about 2 – 3 times only for α-BHC and β-BHC compounds if we compare them with data reported at European level, but if we compare them with results coming from Asiatic countries, our values were approximately in the same range or lower. However, these increased levels of pesticide residues in breast milk samples were recorded mainly in samples of mothers from rural areas. In case of breast milk samples collected from mothers living in urban areas, the detected pesticide residues values align with those reported at European level. Regarding the presence of mono- and polycyclic aromatic hydrocarbon compounds in breast milk samples are just a few studies published to date. As regards from studies performed in

Europe, Zanieri et al., (2007) reported higher values of acenaphthylene, naphthalene, acenaphthene, fluoranthene and pyrene with an average value of these major polycyclic aromatic compounds located between range of 1.4 – 9.09 ng·g^{-1} lipid weight.

Europa

Country	Compounds (ng·g^{-1} l.wt.)							References
	HCB	α-BHC	β-BHC	γ-BH	4.4'-DDD	4.4'-DDE	4.4'-DDT	
Poland (2006)	4.2 – 40.7	n.s	up to 11.1	n.s	n.s	29.4 – 984	26 - 112	Jaraczewska et al., 2006
Poland (2007)	13.7 – 36.7	0.21 – 0.73	10.6 – 33.2	0.17 – 4.5	1.66 – 8.72	493 - 3165	30.2 – 137.6	Szyrwinska and Lulek, 2007
Italy	38 – 70	n.s	n.s	n.s	n.s	210 – 510	9.4 – 44	Abballe et al, 2008
Czech	23.8 - 739	n.s	8.7 - 108	n.s	n.s	3.37 - 2016	7.15 - 264	Cerna et al, 2010
Norway	11 - 27	0.1 – 0.4	6.1 - 36	0.1 – 3	0.1 – 0.8	34 - 278	2.8 - 15	Polder et al, 2008 (a)
Russia	33 - 119	2 - 9	74 - 552	0.5 - 7	1 - 6	428 - 1473	25 - 230	Polder et al, 2008 (b)
Asia								
China (2007)	0.1 – 262.1	0.4 - 117.5	56.8 – 846.7	1.14 – 380.8	n.s.	248.8 – 4700.2	n.s.	Zhao et al, 2007
China (2011)	18.4 – 56.8	up to 8	42 – 552.9	up to 21.6	up to 8.9	140.2 – 1660.4	up to 110.5	Zhou et al, 2011
India	n.s.	2330 – 2720*	n.s	n.s	2870 – 3210**	n.s	n.s	Mishra and Sharma, 2011
Taiwan	n.s.	n.s	up to 7	up to 6	n.s	19 - 1332	6 - 83	Chao et al, 2006
Hong Kong	17 – 29.6	up to 1	288 - 1380	up to 2	n.s	n.s	n.s	Hedley et al, 2010
Philippines	0.76 – 6.9	0.07 – 0.86	0.95 - 17	0.049 – 2.2	0.06 - 11	10 - 2000	1.1 - 35	Malaewannan et al, 2009
Indonesia	0.41 - 7	up to 3.1	0.55 - 130	up to 11	0.19 - 25	14 - 12000	2.2 - 2400	Sudaryanto et al, 2006
Middle Asia								
Iran	20 - 8770	50 – 16 170*	n.s	n.s	70 - 18370**	n.s	n.s	Behrooz et al, 2009
Tunisia	1 - 727	n.s	1 - 285	up to 116	2 – 2461	3 - 6800	1 - 2499	Ennaceur et al, 2008
South America								

Brazil	n.s	n.s	n.s	n.s	n.s	10.7 – 7271.5	3 – 2534.1	Azuredo et al, 2008
Africa								
South Africa (2006)	n.s	n.s	n.s	n.s	2.45 – 955.4	30.2 – 13782.2	24.4 – 9685.9	Bouwman et al, 2006
South Africa (2011)	n.s	n.s	n.s	n.s	n.s	20 – 6000	98 - 13600	Darnerud et al, 2011
Australia								
Australia	6.6 - 76	up to 0.18	10 - 660	0.1 – 0.5	0.06 – 0.45	150 - 870	3.6 - 30	Mueller et al, 2008

*Total HCH; **Total DDT; n.s. – not studied/reported in cited paper.

Table 3. Comparison of organochlorine pesticides, mono- and polycyclic aromatic hydrocarbon levels in human breast milk samples from various countries at worldwide levels.

4.2. Regional distribution of environmental contaminants residues in human breast milk

Mother breast milk monitoring program was designed to compare qualitatively and quantitatively the differences of early age infant exposure to organochlorine pesticides, mono- and polycyclic aromatic hydrocarbons between different regions, urban vs. rural, from north-western part of Transylvania. Comparing the results of average value of all organochlorine pesticides from the total of 169 breast milk samples, it was observed that in case of samples collected from rural regions the organochlorine compounds contaminants quantitative abundance is 80 % than for samples from urban areas where the quantitative abundance is 20 %. In terms of qualitative abundance the results show that 8.32 is the average value of the number of organochlorine compounds detected per samples collected from both regions (n=169) from which the values of 10.11 and values of 6.55 correspond for samples collected from rural (n=80) and urban areas (n=89), respectively. According with table 4, similar behavior was observed even when were compared the maximum and minimum value as well the frequency of detection for each monitored organochlorine compounds separately.

Organochlorine pesticide compounds	Breast milk samples from mothers living in rural area (n=80)			Breast milk samples from mothers living in urban area (n=89)		
	Frequency*	Average	Range	Frequency*	Average	Range
Aldrin	54; 67.5	30.49	2.4-397.3	42; 47.19	5.96	1.2-32.6
Lindane	52; 60	17.9	1.4-141.1	30; 33.7	5.08	1.1-54.5
α-BHC	27; 33.75	18.41	1.9-311.7	31; 34.83	3.21	0.3-22.3
β-BHC	38; 47.5	22.75	1.9-352.4	36; 40.44	4.63	0.7-29.04
δ-BHC	20; 25	5.77	2.7-119.8	25; 28.08	2.67	0.7-64.7
α-Chlordane	39; 48.75	16.92	4.5-171.8	22; 24.71	2.21	0.9-24.65
γ-Chlordane	29; 36.25	13.72	2.8-170.8	13; 14.6	1.1	0.24-26.64
4.4'-DDD	46; 57.5	14.56	1.4-135.4	35; 39.32	3.88	0.64-27.93
4.4"-DDE	61; 76.25	60.79	7.7-291.3	84; 94.38	36.06	2.9-103.6
4.4'-DDT	68; 85	81.03	1.5-791.3	77; 86.51	22.31	0.21-72.89
Diledrin	50; 62.5	29.37	2.3-292.2	38; 42.69	5.43	0.27-50.94
α-endosulfan	48; 60	17.56	3.3-216.4	26; 29.21	1.89	0.3-19.01
β-endosulfan	22; 27.5	3.49	0.9-38.3	19; 21.34	1.61	0.3-26.2
Endosulfan sulfate	43; 53.75	9.28	3.2-85.2	7; 7.86	0.2	0.2-9.2
Endrin	39; 48.75	22.84	0.6-275.6	25; 28.08	4.31	1.07-43.32
Endrin aldehyde	43; 53.75	10.64	2.6-111.1	25; 28.08	3.4	0.79-38.7
Endrin ketone	31; 38.75	8.16	1.9-141.1	19; 21.34	2.07	1.03-25.6
Heptachlor	35; 43.75	19.93	1.5-296.5	19; 21.34	2.26	0.9-28.5
Heptachlor epoxide (isomer B)	34; 42.75	13.77	1.8-160.4	7; 7.86	0.77	0.6-36.9
Methoxychlor	32; 40	10.3	1.9-188.7	2; 2.24	0.02	0.9-1.2

* Frequency – expressed as number of mothers in that the contaminant residue was detected and as percentage (%) from the whole monitored subjects from the corresponding regions (urban, rural regions).

Table 4. Organochlorine pesticides residues distribution in breast milk samples of mothers living in rural and urban regions (Quantitative amount of monitored contaminants from milk samples are expressed in ng·g^{-1} lipid weight).

If we analyze table 5, the same differentiated regional behavior could be observed even in case of mono- and polycyclic aromatic hydrocarbons as in case of organochlorine pesticides, thus the qualitative as well quantitative abundance of them were higher in rural areas (67 %) than in urban regions (33 %). Considering the qualitative abundance for the total mono- and polycyclic aromatic hydrocarbon compounds the results show that 5.727 is the average value of the number of monitored hydrocarbon compounds detected per samples collected from both regions (n=169) from which the values of 5.114 and values of 0.614 correspond for polycyclic aromatic hydrocarbons and monocyclic aromatic hydrocarbon compounds, respectively. The average value for quantitative amount of total polycyclic aromatic hydrocarbon compounds detected per milk samples were 4.651 (ng·g^{-1} lipid w.) and 4.899 (ng·g^{-1} lipid w.) for samples collected from rural and urban regions, respectively, while in case of mono-aromatic hydrocarbon compounds the average values were 0.960 (ng·g^{-1} lipid w.) and 0.823 (ng·g^{-1} lipid w.) for breast milk samples collected from rural and urban regions, respectively. Considering the results presented in table 5, similar behavior was observed even when were compared the maximum, minimum, average values as well the frequency of detection for each monitored hydrocarbon compounds separately.

Polycyclic aromatic hydrocarbons	Breast milk samples from mothers living in rural area (n=80)			Breast milk samples from mothers living in urban area (n=89)		
	Frequency*	Average	Range	Frequency*	Average	Range
Acenaphthene	14; 17.5	1.89	0.52-29.89	26; 29.21	3.36	0.16-30.28
Acenaphthylene	15; 18.75	1.49	3.12-21.38	21; 23.59	1.71	2.13-18.22
Anthracene	8; 10	4.98	2.83-140.52	19; 21.34	2.02	0.64-16.57
Benz[a]anthracene	25; 31.25	6.36	3.54-74.62	29; 32.58	6.11	1.35-57.6
Benz[b]fluoranthene	26; 32.5	6.23	1.81-61.3	32; 35.95	7.25	1.31-110.65
Benz[ghi]perylene	29; 38.25	8.57	1.72-88.91	34; 38.2	8.26	1.83-76.19
Benz[a]pyrene	24; 30	6.75	8.15-71.12	32; 35.95	7.08	0.39-72.75
2-bromonapthalene	11; 13.75	1.99	1.51-59.62	16; 17.97	2.73	1.09-58.48
Chrysene	26; 32.5	5.27	2.58-38.87	35; 39.32	7.26	3.04-91.37
Dibenz[a,b]anthracene	25; 31.25	5.38	2.34-56.77	31; 34.83	5.07	2.23-41.75

Fluoranthene	24; 30	4.1	3.29-47.1	27; 30.33	4.41	3.31-51.52
Fluorene	24; 30	4.13	2.09-31.90	25; 28.08	3.47	2.36-40.44
Indenol [1.2.3-cd]pyrene	22; 27.5	4.41	1.72-46.02	24; 26.96	3.95	1.89-40.83
Naphthalene	36; 45	7.08	3.75-42.01	41; 46.06	7.18	3.11-46.52
Phenanthrene	23; 28.75	2.99	2.36-28.21	28; 31.46	4.04	1.26-91.95
Pyrene	22; 27.5	3.6	2.19-32.03	24; 26.96	4.44	3.72-88.15
Monocyclic aromatic hydrocarbons						
Benzene	7; 8.75	0.89	2.82-35.11	8; 8.98	0.62	2.42-16.29
Ethylbenzene	8; 10	0.72	3.12-22.56	8; 8.98	0.59	4.17-17.42
Toluene	15; 18.75	1.89	3.32-17.19	19; 21.34	1.79	2.41-19.03
m,p-xylene	8; 10	0.86	1.44-26.15	8; 8.98	0.63	1.5-14.55
o-xylene	6; 7.5	0.42	2.15-8.01	8; 8.98	0.46	3.07-6.3

* Frequency – expressed as number of mothers in that the contaminant residue was detected and as percentage (%) from the whole monitored subjects from the corresponding regions (urban, rural regions)

Table 5. Mono- and polycyclic aromatic hydrocarbon compounds residues distribution in breast milk samples of mothers living in rural and urban regions (Quantitative amount of monitored contaminants from milk samples are expressed in $ng \cdot g^{-1}$ lipid weight).

As show in figure 1, for these classes of chemical compounds cannot highlight a strong differentiation between the breast milk samples from mothers living in rural area and those from urban areas.

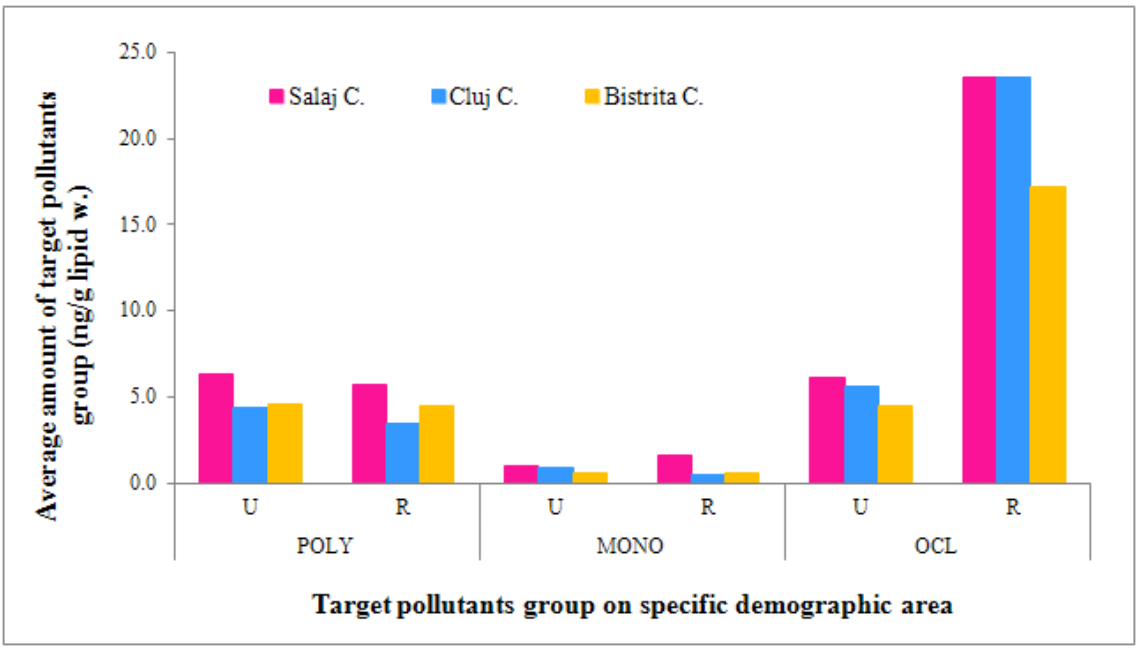

Figure 1. Average amount of detected contaminants in breast milk samples (ng·g^{-1} lipid w.) vs. the corresponding demographic area (Salaj county, Cluj county and Bistrita-Nasaud county). Notation in figure 1: U – urban area; R – rural area; MONO – monocyclic aromatic hydrocarbons; POLY – polycyclic aromatic hydrocarbons; OCL – organochlorine pesticides.

Analyzing the obtained results for each target contaminants class (figure 1), it can be seen that the areas that are the most prone for contamination if we consider the results of contaminants presence in breast milk samples of monitored mothers, are counties Salaj and Cluj whether we refer to rural or urban areas. Also greater contaminations in all regional areas are registered with organochlorine pesticides, especially in case of rural regions.

4.3. Major and minor determining factor that could influence maternal milk contamination status

Previous studies has shown that in case of any nursing mothers there are several factors that could contribute in a positive way to accumulation of environmental contaminants in their organisms, respectively their breast milk (Harris et al., 2001). Some of these major factors are mothers' age, parity, diet and many other external factors as living and workplaces (Ennaceur et al., 2008).

4.3.1. Difference in contaminant residues amount between multipara and primpara mothers

As we know according to previously published studies, there are many factors that could influence in a greater or less proportion the "storage" (existing quantity) of organic pollutants in breast milk samples; one of these factors is the mothers' parity. Through correlation of measured data regarding organic pollutants from breast milks with corresponding mothers' number of children it was observed that in case of organochlorine pesticides higher amount were detected in case of mothers at first birth. This feature was available both for mothers living in urban areas and for those from rural areas, regardless of the district they belonged. Similar behaviour was determined in case of polycyclic aromatic hydrocarbons. This trend of decreasing amounts of pollutants in breast milk once with number of births was motivated by researchers through loss realized on excretion pathways, namely nursing (Sudaryanto et al., 2006; Mishra et al., 2011). Anyway to could consider this assumption as accurate there are needed more precise information regarding breastfeeding period and length of lactation, food frequency and levels of food contaminants, etc., information that we don't have in this study, thus the correlation was made only by comparing the measured pollutants amount from breast milk with number of children of the corresponding mothers. Although this trend was observed both for organochlorine pesticides as well

for polycyclic aromatic hydrocarbons, in case of monocyclic aromatic hydrocarbons any conclusions in this direction couldn't be taken.

4.3.2. Difference in contaminant residues amount considering mother ages and breast milk lipid content

Similar correlation we done in case of organic pollutants detected in breast milk and corresponding mothers age (figure 2) as well lipid content of milk (figure 3). Increases of monitored organic pollutants once with mothers age was observed yet. This tendency could be attributed to bioconcentration capacity of these pollutant species in living organisms. However, when maternal age was correlated with the monitored pollutants amount detected in their breast milk, a better correlation was observed in samples collected from rural areas to those collected from urban area, which can be attributed to the existence of a larger number of disturbing factors (residential pollution, regional pollution, etc.) in case of mothers living in urban areas.

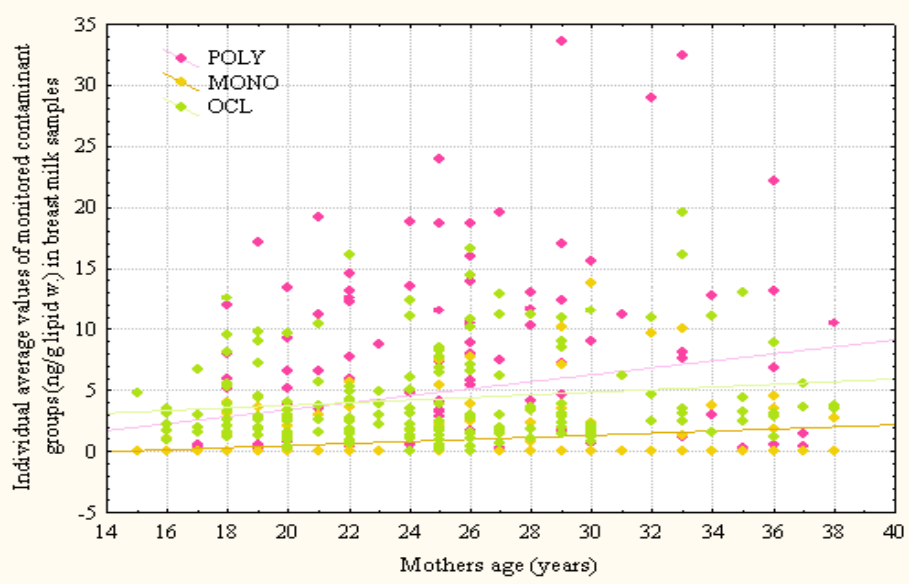

Figure 2. Average amount of detected contaminants in breast milk samples (ng·g^{-1} lipid w.) vs. the corresponding mother age expressed in years at sampling moment.

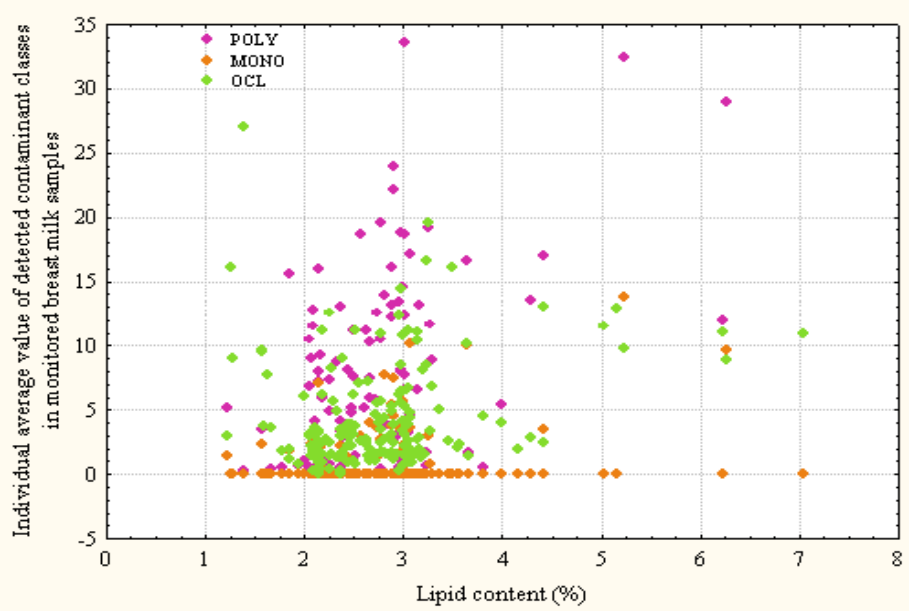

Figure 3. Average amount of detected contaminants in breast milk samples (ng·g^{-1} lipid w.) vs. the corresponding breast milk lipid content.

Notation in figure 2 and figure 3: MONO – monocyclic aromatic hydrocarbons; POLY – polycyclic aromatic hydrocarbons; OCL – organochlorine pesticides.

Considering the results showed through correlation of breast milk lipid content with amount of detected pollutants it was observed that once with increasing of lipid content an increasing of pollutants concentration has achieved (figure 3). Once again, the correlation pattern obtained between monitored pollutants with mothers age and breast milk lipid content was not true for monocyclic aromatic hydrocarbons.

4.4. Infants early age exposure status - Estimated daily intake (EDI)

Although the uses of chemicals as organochlorine pesticides has been strictly regulated or even forbidden, in wide number of compounds cases their remaining residues still continue to affect biota and the surrounding environment. On the other hand this problem is magnified by the fact that although their use is highly regulated in terms of commercially agricultural productions, there are no regulatory legislation in terms of agricultural productions for own consumption. Regarding case of mono- and polycyclic aromatic hydrocarbon compounds the situation is almost similar; even in this case there are legislations that limiting pollution levels coming from industrial sites, the application of these laws to small communities is more difficult. Problems arising from the use and/or unwanted/misunderstood generation of potential toxic chemicals to living organisms together with their authorized production and uses continue to leave a last beneficial impact on biota and thus on humans health, contributing to exposure since early life age – case of neonates exposure.

In order to determine whether there is a potential risk to the ingestion of human breast milk by infants daily intake factor of monitored contaminants were calculated based on the determined amounts of contaminants in breast milk from monitored nursing mothers. Following equation was used for estimated daily intake (EDI) calculation:

$$EDI = \frac{C_{milk} \cdot 700\ g \cdot C_{lipids}}{5\ kg} \qquad \text{(eq.1.)}$$

where C_{milk} represent the amount of contaminant determined in breast milk, C_{lipids} is the amount of lipids from breast milk and 700 g and 5 kg were considered as standard values for parameters as ingested milk quantity and infant body weight, respectively.

Average values and ranges of determined daily intakes compared with the guidelines standard values proposed by ATSDR, WHO and Health Canada (Ennaceur et al, 2008; Baars et al, 2001) are presented in table 6 for monitored organochlorine pesticides and in table 7 for monitored mono- and polycyclic aromatic hydrocarbon compounds.

Organochlorine compounds	ADI*	Mothers from rural region			Mothers from urban region		
		Average	Min EDI	Max EDI	Average	Min EDI	Max EDI
Aldrin	0.1	0.172	0.005	3.418	0.022	0.004	0.133
Lindane	1	0.08	0.003	1.013	0.019	0.003	0.19
α-BHC	0.2	0.093	0.003	2.191	0.012	0.004	0.076
β-BHC	0.04	0.126	0.003	2.477	0.017	0.003	0.112
δ-BHC	-	0.032	0.009	0.78	0.010	0.003	0.276
α-Chlordane	0.5	0.073	0.009	1.241	0.008	0.004	0.088
γ-Chlordane	0.5	0.061	0.01	0.78	0.004	0.001	0.093
4.4'-DDD		0.065	0.007	0.559	0.014	0.002	0.12
4.4"-DDE	0.5**	0.28	0.023	2.286	0.136	0.011	0.516

4.4'-DDT		0.436	0.023	7.799	0.084	0.001	0.277
Dieldrin	0.1	0.15	0.015	2.88	0.02	0.001	0.193
α-endosulfan	6	0.084	0.007	1.057	0.007	0.004	0.077
β-endosulfan	6	0.014	0.003	0.168	0.006	0.001	0.106
Endosulfan sulfate	6	0.044	0.012	0.548	0.001	0.001	0.032
Endrin	0.2	0.121	0.001	1.702	0.015	0.003	0.115
Endrin aldehyde	0.2	0.06	0.012	0.667	0.011	0.003	0.104
Endrin ketone	0.2	0.039	0.004	0.871	0.007	0.004	0.1
Heptachlor	0.1	0.088	0.006	1.345	0.008	0.003	0.115
Heptachlor epoxide (isomer B)	0.1	0.066	0.01	1.065	0.003	0.003	0.149
Methoxychlor	5	0.051	0.004	1.657	0.0001	0.004	0.005

*ADI (acceptable daily intake) expressed as $\mu g \cdot kg^{-1}$ body weight according with values listed by ATSDR, in 1998 and 2005, IPCS 1996, Baars et al, 2001; ** for total DDT the ADI values was set at 20 $\mu g \cdot kg^{-1}$ body weight;

Table 6. Estimated daily intake average value, minim value and maxim value for detected organochlorine pesticides from monitored mothers breast milk

Organochlorine compounds	ADI*	Mothers from rural region			Mothers from urban region		
		Average	Min EDI	Max EDI	Average	Min EDI	Max EDI
Acenaphthene	-	0.01	0.002	0.255	0.012	0.001	0.096
Acenaphthylene	-	0.006	0.009	0.1	0.006	0.007	0.075
Anthracene	50	0.018	0.01	0.594	0.008	0.002	0.066
Benz[a]anthracene	-	0.035	0.01	0.523	0.022	0.004	0.227
Benz[b]fluoranthene	-	0.031	0.008	0.429	0.027	0.004	0.412
Benz[ghi]perylene	30	0.043	0.004	0.651	0.03	0.007	0.266
Benz[a]pyrene	-	0.033	0.02	0.521	0.027	0.002	0.313
2-bromonapthalene	-	0.01	0.006	0.437	0.012	0.003	0.185
Chrysene	50	0.025	0.008	0.341	0.028	0.01	0.372
Dibenz[a,b] anthracene	-	0.027	0.009	0.303	0.019	0.008	0.179
Fluoranthene	50	0.019	0.012	0.211	0.015	0.016	0.208
Fluorene	40	0.021	0.005	0.234	0.013	0.01	0.165
Indenol [1.2.3-cd]pyrene	-	0.021	0.004	0.337	0.015	0.006	0.175
Naphthalene	40	0.034	0.007	0.369	0.027	0.01	0.207
Phenanthrene	40	0.015	0.007	0.248	0.015	0.004	0.375

Pyrene	-	0.019	0.005	0.198	0.015	0.011	0.253
Monocyclic aromatic hydrocarbons							
Benzene	4.3	0.006	0.006	0.308	0.003	0.007	0.066
Ethylbenzene	100	0.003	0.008	0.115	0.002	0.014	0.069
Toluene	223	0.008	0.012	0.106	0.007	0.007	0.082
m,p-xylene	150	0.005	0.006	0.191	0.002	0.006	0.059
o-xylene		0.002	0.006	0.057	0.002	0.011	0.026

*ADI (acceptable daily intake) expressed as $\mu g \cdot kg^{-1}$ body weight according with values listed by ATSDR, in 1998 and 2005, IPCS 1996, Baars et al, 2001; ** for total DDT the ADI values was set at 20 $\mu g \cdot kg^{-1}$ body weight;

Table 7. Estimated daily intake average value, minim value and maxim value for detected mono- and polycyclic aromatic hydrocarbons from monitored mothers breast milk

Comparing the calculated EDI for the target organic pollutant classes it was observed that in case of organochlorine pesticides the acceptable daily intake value was exceeded significantly in many cases as aldrin, β-BHC, DDE, DDT, endrin, heptachlor and heptachlor epoxide. No exceeding of settled ADI values was achieved in case of mono-and polycyclic aromatic hydrocarbons.

These results showed that ignorance regarding organochlorine pesticides presence in surrounding environment and its impact on living beings must to be avoided as much as possible.

5. CONCLUSIONS

As we noted after consulting the literature, this presented study is the first that present the contamination status with organochlorine pesticides, mono- and polycyclic aromatic hydrocarbon residues in human breast milk from Transylvanian regions of Romania. In breast milk samples collected from a totally 169 nursing mothers organochlorine pesticides were detected in higher amount and number, strong qualitative and quantitative presence of organochlorine pesticides being registered in case of samples coming from rural areas. As regards mono- and polycyclic aromatic hydrocarbons in about 42 % of milk samples at least one of these compounds was detected but their levels being lower that case of organochlorine pesticide compounds. Correlation between detected pollutants amount from breast milk and mothers' characteristics were done also showing that mothers age and their milk lipid content posing positive effects on pollutants accumulation, excepting cases of monocyclic aromatic hydrocarbon pollutants cases. Estimated daily intake value of monitored pollutants in case of infants exposure was evaluated also, showing that in case of a large number of organochlorine pesticides these values exceed the acceptable daily intake established through legislations. As regards mono- and polycyclic aromatic hydrocarbons no exceeding of acceptable daily intake values was determined.

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