



Evaluation PAHs Contamination Level in the Breast Milk of Women in Al-Diwaniyah city, Iraq- a Case Study

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Abstract

Human milk is an important bio indicator for the exposure with some pollutants like polycyclic aromatic hydrocarbons. Breast milk samples from 68 women were live in Al-Diwaniyah city were collected in this study. Thirteen of poly cyclic aromatic hydrocarbons were investigated in all samples using gas chromatography coupled with mass spectroscopy. The samples were divided into three groups according to the age range (less than 20 years, 20-30 years and 31-40 years). Eight samples were the control for this study from the rural mothers. The mothers were all them non-smokers. The samples were collected during 2017 from different regions located in Al-Diwaniyah City.

Keywords: *Breast milk, Polycyclic aromatic hydrocarbons, Pollution in Al-Diwaniyah City, Gas Chromatography, Exposure to pollutants.*

Introduction

Polycyclic aromatic hydrocarbons are consisting of two or more fused aromatic rings [1]. These compounds are come in an environment either by incomplete burning of organic materials or petrogenic origin [2]. PAHs are occurred in food as a result for the both sources petrogenic and pyrolytic origin [3]. The main source of feeding for the children within first months after birth is breast milk due to the content of this milk which consist of the main elements for growing healthy for the babies [4]. The human activities in an environment causes of this environment polluted with different pollutants one of them are PAHs compounds.

Breast milk considers as a perfect bio indicator for pollution with these compounds. Exposure for these pollutants may be in direct methods like smoking, breathing of industrial smoke, grilling smoke and burning smoke which intake via breathing or via drinking and eating polluted water and food. A lot of techniques have been utilized in determination of these compounds in several polluted samples such as Gas Chromatography [5], GC-MS/MS [6], Gas Chromatography coupled to high Mass Spectrometry (HRGCHRMS) [7], HPLC/UV-Vis-DAD/MS [8], Gas Chromatography [9], High Performance Pressure Chromatography

[10]. Several agencies in word have been classified polycyclic aromatic hydrocarbons to several classes according to the degree of their severity. The international agency for research on cancer was classified these compounds into three groups; the first one as carcinogenic action for the both human and animals, it was named class one, while the rest of these compounds it was named class 2A and 2B [11]. Contaminated milk with PAHs depends on ecosystem related to the rearing factors such as contaminated soil, location of exposure to the pollutants in air during inhalation or absorption by dermal contact.

The produced milk of mammary gland can deliver the pollutants like PAHs to the babies during feeding process. For this reason milk considers as biomarker for the infection of mothers with different pollutants [12]. Al-Diwaniyah is a province located in the mid of Iraq. This city was reported a lot of infection with cancer in different parts of body. The potential PAHs production, exposure process, and health risks in Al-Diwaniyah city is take little consideration by the researchers, for this reason this paper focused about exposure of the mothers to the thirteen PAHs by assessment of these compounds in breast milk to evaluate this carcinogenic potential

for these compounds. The objective of this paper is to evaluate the exposure to the polycyclic aromatic hydrocarbons by several human potential in an environment by analysis the breast milk samples from women they are live in Al-Diwaniyah city, Iraq.

Materials and Methods

Reagents and Materials

All reagents and chemicals used in this study were analytical grade. Standard solution of EPA 525 polycyclic aromatic hydrocarbons mixture was supplied from Supelco Company.

This mixture consist of thirteen of polycyclic aromatic hydrocarbons were: Benzo(a)pyrene (BaP), Phenanthrene (Phen), flouremthene (F), Benz (a) anthracene (BaA), Anthracene (A), Pyrene (Py), Fluorene (Fl), Benzo (b) flouremthene (BbF), Indeno (1,2,3-cd)pyrene (IcdPy), Benzo (ghi) perylene (BghiP), Benzo(k) fluoranthene (BkF), Chrysene (ChR) and Dibenz (a,h) anthracene (DBahA).

Table 1 shows the high and low molecular weight of 13 compounds of PAHs. Solvents were obtained from Sigma-Aldrich, anhydrous sodium sulfate purchased from BDH Company.

Table 1: List of 13 analyzed PAHs

Low Molecular weight petrogenic PAHs	High Molecular weight pyrolytic PAHs
A	Icdby
Fl	BghiP
Phen	DBahA
	BaP
	BkF
	BbF
	ChR
	BaA
	Py
	F

Sample Collection and Preparation

Sixty eight samples of breast milk were collected during the period October 2017 to March 2018 from the mothers living in Al-Diwaniyah City. A control locality in the western of AL-Diwaniyah City in small city Al-Dagharah, eight samples during the period February 2018 to March 2018 were collected as a control samples. About 20 mL of human milk were collected from each mother in cleaned glasses amber bottles and kept at temperature -20 °C. The age of target

women was ranged between 18 to 40 years old. Several parameters were take in consideration for the women such as all the donors women are non-drinking of alcohol, non-smoker and healthy in addition to other information were investigated such as (weight, height, number of pregnancies, working with field rich with production of PAHs or not, living in location near the pollution sources, deal with petroleum derivatives or coal and if it was bake the bread in her home, all these obtained from questioners as shown in Table 2.

Table 2 Characteristics of donor mothers, values are presented as mean ± standard deviation

Age/ years	28 ±3.9
Weight/ Kg	63 ± 4.4
Hight/ cm	164 ± 5.1
No. of pregnancies	2 ± 0.7
Pregnancy period/ week	39 ± 5.7
Working in polluted weather	3 ± 1.3
Living in polluted region	5 ± 1.2
Deal with polluted materials	2± 0.6
Bread baker	8 ± 1.5

Extraction of PAHs in Breast Milk

Thirteen of polycyclic aromatic hydrocarbons in breast milk samples were extracted using

the solid phase extraction column method supplied by Supl clean™ ENVI-18 SPE Tube bed weight. 500 mg, volume of tube 6 mL, pkg of 30 ea. Tube of extraction was conditioned with several solvents to increase the efficiency of extraction process. These solvents they were Acetonitrile, Dichloromethane and Ethanol. After conditioning of extraction column A 5 mL of liquid milk sample were mobilized into cleaned bottle made of PP. Acetonitril was added (10) mL to the bottle with milk sample, then vortexed for 1 minute. 1 gm of anhydrous sodium sulfate was added to the mixture. The mixture then was centrifuged for 10 min with 4000 rpm. Organic layer was transferred and passing nitrogen gas to reduce the volume of the sample to 1 mL.

Instrumentation (GC Analysis)

A set of diluted solutions were prepared to get the calibration curves for every compounds of 13 under consideration. A 1 µL of sample were injected in GC 2010 (Shimadzu, Japan). The following conditions details were adjusted during analysis: column type for GC was Hp5 with dimension (60m*0.25mm*0.25µm), injection volume for the sample 1 µL, injector mode split less, temperature of injector 250 °C, carrier gas high purity of nitrogen and detector type flame ionization detector. The retention time for every compound were : Fluorene 8.1, Phenanthrene 8.4, Anthracene 8.8, Fluoranthene 9.9, Pyrene 10.7, Benzo(a) anthracene 12.1, Chrysene 16.3, Benzo (b) fuoranthene 18.0, Benzo (k) fuoranthene 18.6, Benzo (a) pyrene 21.2, Dibenzo (a,h) anthracene 22.5, Benzo(ghi)pyrelene 24.4 and Indeno1,2,3-cd)pyrene 32.3 min.

Results and Discussion

High differences in levels of polycyclic aromatic hydrocarbon were detected in breast milk during previous studies [13, 14]. Literatures were reported that the highest levels in low M. Wt of PAHs [15].

Detection of high levels for these compounds due to the physicochemical properties for these compounds in addition to the origin of these compounds which came from petrogynic sources which may be the donor mother was deal it before collection the saples. These properties may contribute to their distribution in breast milk samples. For instance Phenanthrene is mobilized to the breast milk in human faster with highest concentrations due to the mammary transportation of polycyclic aromatic hydrocarbons which is inversely related to value of M. Wt [16]. Previous studies were reported that the main cause of elevate the concentrations of Anthracene is related to the abundance of Anthracene in polycyclic aromatic hydrocarbons [17].

While the elevation of concentrations for high molecular weight of polycyclic aromatic hydrocarbons in human breast milk may be attribute to kind of pollutants that spread in an environment of study area. The main source of high molecular weight of polycyclic aromatic hydrocarbons is pyrolytic process in an environment, for this reason it's normally occur these compounds in high concentrations relative to low molecular weight of PAHs. The human activities are significant in our environment which release high quantities of pollutants like PAHs. This factor may be refers to the elevation of concentrations of high M.Wt of PAHs in urban centers [18]. Table 3, shows the detected concentrations of polycyclic aromatic hydrocarbons in three groups were classified according to age of the donor mothers live in Al-Diwaniyah City.

These groups are distributed to three classes, the class A which include the women that in age bellow twenty years old, class B have the range of age between twenty to twenty nine years old, while the third class C consist of mothers those in range between thirty to thirty nine years old.

Table 3: PAHs concentrations (mg.kg⁻¹) of breast milk for the donor women

PAHs compound	Group A		Group B		Group C	
	Mean	Range	Mean	Range	Mean	Range
Fluorene	ND	-	ND	-	1.2	0.8-1.7
Phenanthrene	1.1	0.9-2.1	1.4	1.2-2.3	1.2	0.5-1.9
Anthracene	ND	-	ND	-	ND	-
Fluoranthene	ND	-	ND	-	2.3	1.6-3.4
Pyrene	ND	-	ND	-	ND	-
Benzo(a)anthracene	1.8	1.3-2.8	2.6	0.9-3.3	2.8	0.6-3.5
Chrysene	ND	-	ND	- ¹	ND	-

Benzo(b)fluoranthene	1.2	0.4-2.4	1.3	1.0-2.7	1.8	1.4-2.8
Benzo(k)fluoranthene	ND	-	2.2	1.3-3.2	2.6	1.0-4.1
Benzo(a)pyrene	1.9	1.5-2.4	2.2	2.0-3.2	2.5	0.9-4.2
Dibenzo(ah)anthracene	ND	-	1.6	0.7-2.6	ND	-
Benzo(ghi)perylene	0.8	0.6-1.3	1.8	0.9-3.1	1.7	0.9-2.9
Indeno(1,2,3-cd)pyrene	ND	-	ND	-	ND	-

In this research as illustrated in table 3, the polycyclic aromatic hydrocarbons from the pyrolytic sources are much higher than those from petrogenic sources. Several reasons follow this difference in the detected concentrations which may be attributed to the emission of polluted gases as a result for high traffic volume. From table.3, the data illustrate (mean, maximum and minimum value) for every compound under investigation for the three groups.

The low molecular weight of PAHs (Fluorene, Phenanthrene and Anthracene), were with lowest concentration in three studied groups comparison with totally higher molecular weight (Fluoranthene, Pyrene, Benzo (a) anthracene, Chrysene, Benzo(b)fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene, Dibenzo (ah) anthracene, Benzo (ghi) perylene and Indeno (1,2,3-cd) pyrene) of detected PAHs.

This result indicates the spread of pyrolytic origin of PAHs in an environment for area study compare with detected concentration of the PAHs that subjected to petrogenic origin. The results show that the group C is most affected by the polluted environment 8 of 13 compounds was detected in this group. Group B were 7 of 13 compounds and group A was the lowest group affected by the environmental weather were only 5 of 13 compounds detected in this group. Generally the samples of old ages group (C) are the highest affected in the number of detected compounds and the values of measured concentrations of PAHs.

The most abundant representative measured concentration was for Benzo (a) anthracene

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in three groups which was (1.8, 2.6 and 2.8) mg.kg⁻¹ for the groups A, B and C respectively. In other hand the compounds [Anthracene, Pyrene, Chrysene and Indeno(1,2,3-cd)Pyrene], no detection for all groups. Compare between this study with previous studies from different countries we can get the following date; all studies were non systematic in the number of samples and number of targeted PAHs under investigation [19]. The effect of smoking was clear on the increasing of detected concentration about five time's more than non-smoking donor mothers [17]. Urban region was significant in the level and number of detected PAHs compare with rural region

Conclusions

The results of study showed the assessment of the impact of age of the donor women in the percentage of pollution with PAHs in Al-Diwaniyah City, Iraq. We can conclude that the success of extraction and analysis method using GC instrument for detect a low concentration of PAHs in measured samples of human breast milk. The compounds Anthracene, Pyrene, Chrysene and Indeno (1,2,3-cd)Pyrene were non detected in all 68 samples of breast milk. Higher molecular weights of PAHs were higher in the abundance than lower molecular weight.

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