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Monitoring of Carcinogenic Environmental Pollutants in Women's Breast Milk

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This study was to investigate polycyclic aromatic hydrocarbons (PAHs), lead (Pb), and cadmium (Cd) levels in women's breast milk. One hundred and twenty milk samples were collected from lactating women who were living around petrochemical factories and a sugar cane factory. The residues of PAHs in women's breast milk samples were determined using gas chromatography and PAH standards after extraction and saponification. Lead and cadmium levels were determined in the milk samples after digestion using an atomic absorption spectrophotometer. Results revealed that different levels of acenaphthylene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, indeno(1,2,3-cd)pyrene, and benzo(g,h,i)perylene were detected in the women's breast milk. The total PAHs were significantly ($P<0.05$) increased in samples from women who were livingaround petrochemical factories and the sugar cane factory. The total carcinogenic PAH levels were significantly ($P<0.05$)increased (4.541±1.643 ng/ml) in samples from women living around the petrochemical factories than they were in samples from women living around thesugar cane factory (0.106±0.026 ng/ml). Lead and cadmium were detected in the milk samples collected from the studied areas but their levels were markedly higher in the samples from women living around the petrochemical factories (0.180±0.014 ng/ml). In conclusions, different compounds and levels of PAHs, Pb, and Cd could be detected in the breast milk samples of women who live in polluted areas.

Keywords: Women's breast milk; Environmental pollutants; Polycyclic aromatic hydrocarbons; lead; cadmium.

In recent years, the amount of environmental pollutants has increased in all industrialized countries. The majority of these pollutants in food originate from precipitated particulate materials. The first report on the presence of the toxic chemical; dichlorodiphenyltrichloroethane (DDT) was published in the 1950s[1]. Breastfeeding is considered to be a defence factor that provides nutrients for appropriate growth and for physical, emotional and immunological development [2].

Human breast milk may contain many lipophilic persistent organic pollutants (POPs), such as heavy metals, polychlorinated dibenzo-p-dioxins, polybromide, hexachlorocyclohexane,

polycyclic aromatic hydrocarbons (PAHs) and pesticides[3]. Serious effects may be associated with the presence of POPs in human breast milk that are readily transferred to infants and are easily absorbed[1].

Polycyclic aromatic hydrocarbons (PAHs) originate from the incomplete combustion of fuel or from the high-temperature pyrolysis of oils and fats[4]. PAHs are environmentally persistent due to their chemical stability and resistance to biodegradation. Reports have shown that the exposure of humans to environments containing PAHs may induce lung and skin cancers[5]. PAHs can cause the development of arteriosclerosis[6], intrauterine growth retardation[7], and newly born's neurological development[8]. In addition, PAHs can bioaccumulate in adipose tissues and are magnified through the food chain due to their lipophilic properties[9].

Lead (Pb) is one of the most dangerous pollutants due to its extensive distribution throughout the environment[10]. Lead is stored mainly in the bone matrix. Lactation increases bone turnover and increases the amount of lead in the plasma and, consequently, in human milk[11]. Chronic intrauterine exposure to lead may lead to growth retardation, neurological defects, attention deficits, and behavioural changes in later stages of life[12].

Cadmium (Cd) is a toxic substance that is released into the atmosphere by coal burning[13]. Cadmium is used in certain industrial products, such as nickel-Cd batteries, paint materials, and stabilizers; it provides heat and light resistance to polyvinyl chloride and metal alloys[14]. Smoking tobacco is an important source of exposure because the tobacco plant naturally absorbs high amounts of Cd in its leaves. Higher concentrations of Cd have been detected in many foods[15]. The main organs that are affected by chronic Cd toxicity are the kidneys, bones and lungs. However, Cd has toxiceffects in almost all body systems.

Inpreviouslystudy, we detected some environmental pollutants in raw cow's milk, but the measurement of such pollutants in the breast milk reflects both the prenatal and postnatal exposure to these chemicals[16].

Therefore, this study was conducted to investigate the levels of polycyclic aromatic hydrocarbons, lead and cadmium residues in the milk samples collected from lactating women who live aroundthe industrial areas ofPetrochemical and Sugar Canefactories.

MATERIALS AND METHODS

This study was approved by the ethics committee of the Aswan University Hospital. All mothers involved in this study were informed about the purpose of the study, and the informed consent agreement declares that these samples will not be used in future research or other studies.

Locality

The study areas were Kema Zone, Kom-Ombo, and Edfu, Aswan Governorate, Upper Egypt. The Sugar Cane Factory is located in Kom-Ombo, and the other Petrochemical Factories are located in Kema Zone in Aswan City.

Data collection and sampling

In this current study, all participants are healthy, lactating mothers who were living in Kema Zone, Kom-ombo, Edfu, in Aswan Governorate for at least 3 years before sample collection. The mothers gave birth to healthy newborns without complicated pregnancies or deliveries. The data collections are illustrated in Table 1. The participating mothers were aged from 26.27±5.53 to 30.76±4.71 years old, and weighted from (55.06±7.32 to 69.36±7.95 kg). The following criteria were collected: education (college/high school), occupation (working/housewife) and smoking (yes/no) and were (15/25, 17/23 and 18/22), (12/28, 13/27 and 12/28) and (4/36, 6/24 and 3/37), respectively in the cities of Kema Zone, Kom-ombo, Edfu.

One hundred and twenty samples of women's breast milk were collected (40 samples from each locality). The samples were collected from March 2015 to February 2016. Sampling collection was carried out in the early morning to the first feeding of the day during the first 6 months of lactation. Approximately 15 ml of milk was collected from each woman into cleaned, sterilized Falcon polypropylene tubes, which had been washed with diluted nitric acid and then rinsed with double distilled water. The milk samples were stored at -20 °C until the analytical process.

Analytical determination

All procedures were carried out in the Department of Forensic Medicine and Toxicology,

Faculty of Veterinary Medicine, Benha University.

The Institutional Ethics Committee approved all experimental protocols (No. 133 at October 2015) that were conducted by the Department of Forensic Medicine and Toxicology, by the Faculty of Veterinary Medicine, Benha University, Egypt.

Determination of polycyclic aromatic hydrocarbons (PAHs)

Detection of PAH residue in milk was performed according to Hegazy *et al*.[16] using standard curve of PAHs which included naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b) fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd) pyrene, and benzo(g,h,i)perylene.

Detection of PAH residue in milk

A gas chromatograph (Hewlett-Packard HP 5890 series II Plus) equipped with a flame ionization detector and an HP-1 analytical capillary column (30 m×0.53 mm), a film thickness (0.88 µm), and a cross-linked methyl silicone gum for the separation of PAHs. The operational conditions of gas chromatography were as follows: detector temperature (280 °C), injection port temperature (200 °C), column initial temperature (100 °C), initial time (2 min), rate of temperature increase (6 °C /min), upper temperature (280 °C) and upper time (10 min). The flow rate of the carrier gas (nitrogen) was 4 ml /min. and the injection volume was 1 µl throughout.

Determination of lead and cadmium

Determination of lead and cadmiumwas performed according to Ismail *et al*[17] using a Perkin-Elmer 2380 Atomic absorption spectrophotometer.

Statistical analysis

The data were subjected to statistical analysis, including the calculation of the mean and standard error (Mean±SE). The differences between groups were tested for significance using a one-way analysis of variance followed by Duncan's Multiple Range test. The differences were considered significant at $P<0.05$using Statistical Package for Social Science (SPSS) software version 20.0 computer programme.

RESULTS AND DISCUSSION

Women's breast milk is the best natural source for feeding new-born infants which contains the correct balance of fats, carbohydrates, and proteins needed for developing babies. Breast milk provides infants with strong immune factors that help them fight diseases[1]. Infants are more sensitive to environmental pollutants than adults due to their low body weight, the rapid growth and development of their nervous system, and easier intestinal absorption[18]. Breast milk provides information about the exposure of the mothers and their infants to such environmental pollutants[19].

The present study revealed that there were different concentrations of PAHs detected in the milk samples (Table 2). Many PAH compounds were detected in the milk samples from women living inKema Zone and Kom-Ombo. Only three compounds of PAHs were detected in the milk samples fromEdfu. Fluorene, anthracene, fluoranthene, and benzo(b) fluoranthene were detected in the milk samples from both Kema Zone and Kom-Ombo. Chrysene was detected in the milk samples from women living in the three studied areas. Pyrene and benzo(g,h,i)perylene were detected in the milk samples fromKema Zone only. Acenaphthylene was detected in the milk samples from Kom-Ombo and Edfu. Phenanthrene and benzo(a)anthracene were detected in the samples fromKom-Ombo only. Indeno(1,2,3-cd)pyrene was detected in the milk samples from Kema Zone and Edfu. Naphthalene, acenaphthene, and benzo(a) pyrene were not detected in the samples from the three studied areas.

The total PAHs were significantly ($P<0.05$) higher in the milk samples from Kema Zone and Kom-Ombo. The lowest level of total PAH was detected in the milk samples from Edfu. The total carcinogenic PAHs were significantly ($P<0.05$) higher in the samples from Kema Zone, while the total non-carcinogenic PAHs were significantly ($P<0.05$) higher in the milk samples from Kom-Ombo (Table 2). This finding may be due to the environmental pollution in the first two studied areas rather than in the pollution in the third area. These results agree with Kim *et al*[20]. The differences in concentrations can be attributed to exposure to these pollutants (indoor and outdoor air exposure), smoke exposure, a diet containing variable amounts of PAHs, broiled food, the study area, the ignition of petroleum compounds, occupational exposures, and the

method of analysis[20]. Lipid-soluble carcinogens may accumulate in the breasts due to the presence of a high amount of adipose tissue. Therefore, the epithelial cells lining the mammary ducts, or those that are suspended in adipose tissue, are susceptible to DNA damage[22]. The higher molecular weight PAH compounds, including benz(g,h,i)perylene, are probable human carcinogens. Recent reports have correlated *in utero* PAH exposure with low birth weight[23], endocrine-disrupting effects, and an impact on female fertility[24]. The health hazard of fluorene reduces red blood cells, packed cell volume and haemoglobin levels. Fluoranthene caused nephropathy, hepatomegaly, haematological alterations, and clinical effects; pyrene is characterized by renal tubular damage[20].

The present study revealed that lead and cadmium were detected in the breast milk samples collected from women in the three studied areas(Table 3). The lead concentration was significantly (P<0.05) higher in the samples from Kema Zone. There were no significant differences in cadmium detected in the three studied areas. These findings agreed with those of Isaac *et al*.[25] stated that mothers living in urban regions contain high lead levels in their milk in India.

In breast milk, the acceptable range of Pb is from 2-5 ng/g according to the WHO[26]. Babies are highly sensitive to the effects of such pollutants due to many factors, including the high rate of gastrointestinal absorption, decreased efficacy of the blood-brain barrier against toxic metals,

Table 1. Characters of the mothers living in different localities in Aswan governorate (Mean ± SE)

Items	Kema zone	Kom-ombo	Edfo
Mother's age	30.761±4.711[a]	26.683±4.632[a]	26.267±5.532[a]
Body weight	55.061 ±7.323[a]	64.543±6.611[a]	69.360±7.951[a]
Educational level(College / High school)	15 / 25	17 / 23	18 / 22
Occupation(Working / Housewife)	12 / 28	13 / 27	12 / 28
Residues in a risk area(Yes or No)	yes	Yes	yes
Smoking (Yes or No)	4 / 36	6 / 34	3 / 37

Means with different letters in the same row are significantly different at P<0.05.

Table 2. Polycyclic aromatic hydrocarbons (PAHs) compounds were detected in human breast milk (ng/ml) collected from different localities in Aswan governorate. (Mean± SE)

PAHs	Kema zone	Kom-ombo	Edfo
Naphthalene	ND	ND	ND
Acenaphthylene	ND	1.844±0.355[a]	0.716±0.249[b]
Acenaphthene	ND	ND	ND
Fluorene	0.380±0.073[a]	0.016±0.003[b]	ND
Phenanthrene	ND	0.222±0.049 [a]	ND
Anthracene	0.293±0.056[b]	2.287±0.784[a]	ND
Fluoranthene	0.200±0.040[a]	0.242±0.053[a]	ND
Pyrene	0.249±0.062[a]	ND	ND
Benzo(a)anthracene*	ND	0.035±0.013[a]	ND
Chrysene*	0.029±0.015[a]	0.024±0.006[a]	0.026±0.001[a]
Benzo(b)fluoranthene*	0.117±0.050[a]	0.047±0.009[b]	ND
Benzo(a)pyrene*	ND	ND	ND
Indeno(1,2,3-cd)pyrene*	0.185±0.071[b]	ND	0.789±0.274[a]
Benzo(g,h,i)perylene*	4.210±1.542[a]	ND	ND
Total PAHs	5.663±1.829[a]	4.717±0.953[a]	1.531±0.524[b]
Total carcinogenic PAHs*	4.541±1.643[a]	0.106±0.026[b]	0.815±0.275[b]
Total non-carcinogenic PAHs	1.122±0.197[b]	4.611±0.962[a]	0.716±0.249[b]

Means with different letters in the same row are significantly different at P<0.05.
* = Carcinogenic PAHs (US-EPA, 2000). ND. = not detected.

Table 3. The level of lead and cadmium (ng/ml) that were detected inhuman breast milk collected from different localities in Aswan governorate. (Mean ± SE)

	Kema zone	Kom-ombo	Edfo
Lead	0.180±0.014[a]	0.016±0.009[b]	0.036±0.013[b]
Cadmium	0.155±0.017[a]	0.084±0.005[a]	0.109±0.024[a]

Means with different letters in the same row are significantly different at $P<0.05$.

immature development of the central nervous system and the detoxification system, and high growth rate of organs and the immune system[27]. In the present study, higher breast milk Pb levels were observed in polluted urban centres, such as Kema Zone compared to those observed in less polluted areas (Kom-Ombo and Edfu). Similar results were recorded by Leotsinidis *et al*.[28] who found higher breast milk Pblevels in mothers residing in city centres than in urban areas.

A high level of Cd could decrease the amount of calcium secreted into the breast milk, indicating maternal Cd exposure[29]. The major sources of cadmium exposure are a diet resulting from contaminated water and crops grown on polluted soil and smoking[30]. Excessive Cd exposure causes disturbances in calcium metabolism and in the formation of renal stones. Additionally, living or working in Cd-contaminated areas may lead to the softening of bones and osteoporosis, osteomalacia, painful bone fractures and renal damage due to Cd accumulation in the bones[30].

CONCLUSION

Different compounds of PAHs, Pb, and Cd could be detected in the breast milk samples collected from lactatingwomen who live near or in the polluted industrial areas. Further investigations of these pollutants should be performed in the blood of newborn infants and in the air, water, plants, and soil of these localities.

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REFERENCES

1. Van den Berg,M., Kypke, K., Kotz, A., Tritscher, A., Lee, S. Y., Magulova, K.,Fiedler, H., and Malisch, R. WHO/UNEP global surveys of PCDDs, PCDFs, PCBs and DDTs in human milk and benefit–risk evaluation of breastfeeding. *Arch Toxicol,* **91**(1): 83-96 (2017). https://doi.org/10.1007/s00204-016-1802-z.
2. Andrade, M.T.S., Del Ciampo L.A., Del Ciampo I.R.L., Ferraz I.S., Ricco R.G., Silva P.S., and Barbosa Júnior F. Determination of lead concentration in breast milk and blood of lactanting women in an interior city of Brasil. *International Journal of Nutrology*, **6**(3):95–100 (2013).
3. Tanabe, S.,and Kunisue, T. Persistent organic pollutants in human breast milk from Asian countries. *Environ. Pollut.,* **146**(2):400–413 (2007).
4. Hitzel, A., Pöhlmann, M., Schwägele, F., Speer, K.,and Jira, W. Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in meat products smoked with different types of wood and smoking spices. *Food Chem.*; **139**(1–4):955–962 (2013).
5. Abdel-Shafy, H.I.,andMansour, M.S. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. *Egypt. J. Petrol.,* **25**(1): 107–123 (2016).
6. Li, X., Yang, Y., Xu, X., Xu, C.,and Hong, J. Air pollution from polycyclic aromatic hydrocarbons generated by human activities and their health effects in China. *J. Clean Prod.,* **112**:1360–1367 (2016).
7. Šrám, R.J., Binková, B., Dejmek, J.,and Bobak,

M. Ambient air pollution and pregnancy outcomes: a review of the literature. *Environ. Health Perspect.,* **113**(4):375–382 (2005).

8. Perera, F.P., Li, Z., Whyatt, R., Hoepner, L., Wang, S., Camann, D., and Rauh, V. Prenatal airborne polycyclic aromatic hydrocarbon exposure and child IQ at age 5 years. *Pediatrics,*2009;124(2):e195–e202.https://doi.org/10.1542/peds.2008-3506.
9. Grimmer, G.*Environmental Carcinogens: Polycyclic Aromatic Hydrocarbons*. CRC Press, (2018).
10. Örün, E., Yalçýn, S. S., Aykut, O., Orhan, G., Morgil, G. K., Yurdakök, K., and Uzun, R.Breast milk lead and cadmium levels from suburban areas of Ankara. *Sci. Total Environ.,* **409**(13):2467–2472 (2011).
11. Lanphear, B.P., Hornung, R., Khoury, J., Yolton, K., Baghurst, P., Bellinger, D. C., Canfield, R. L., Dietrich, K. N., Bornschein, R., Greene, T., Rothenberg, S. J., Needleman, H. L., Schnaas, L., Wasserman, G., Graziano, J., and Roberts, R. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ. Health Perspect.,* **113**(7):894–899 (2005).
12. Abadin, A., Ashizawa, A., Stevens, Y. W., Llados, F., Diamond, G., Sage, G., Citra, M., Quinones, A., Bosch, S. J., and Swarts, S. G.*Toxicological Profile for Lead*. US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta (GA) pp 582, August 2007. https://www.ncbi.nlm.nih.gov/books/NBK158766/pdf/Bookshelf_NBK158766.pdf
13. McConnell, J.R., and Edwards, R. Coal burning leaves toxic heavy metal legacy in the Arctic. *Proceedings of the National Academy of Sciences,* **105**(34):12140–12144 (2008).
14. Elinder, C. Cadmium as an environmental hazard. *IARC Sci.Publ.,* **118**: 123–132 (1992).
15. Yurdakok, K. Lead, mercury, and cadmium in breast milk. *Journal of Pediatric and Neonatal Individualized* Medicine (JPNIM), **4**(2):e040223 (2015). https://doi.org/10.7363/040223.
16. Hegazy, A. M., Khalifa, M. I., and Nasr, S. M. Monitoring of Carcinogenic Environmental Pollutants in Raw Cows' Milk.*Biomed Pharmacol J*, **12**(1): 435–442 (2019).
17. Ismail, A., Riaz, M., Akhtar, S., Goodwill, J. E.,and Sun, J. Heavy metals in milk: global prevalence and health risk assessment. *Toxin Rev,* **38**: 1–12 (2017). https://doi.org/10.1080/15569543.2017.1399276
18. Cinar, N., Ozdemir, S., Yucel, O., and Ucar, F. In which regions is breast-feeding safer from the impact of toxic elements from the environment? *Bosn J Basic Med Sci,* **11**(4): 234–239 (2011).
19. Dong, X., Wang, Q., Peng, J., Wu, M., Pan, B., and Xing, B. Transfer of polycyclic aromatic hydrocarbons from mother to fetus in relation to pregnancy complications. *Sci. Total Environ,* **636**: 61–68 (2018).
20. Kim, S.R., Halden, R.U., and Buckley, T.J. Polycyclic aromatic hydrocarbons in human milk of nonsmoking US women. *Environ. Sci. Technol,* **42**(7):2663–2667 (2008).
21. Pulkrabova, J., Stupak, M., Svarcova, A., Rossner, P., Rossnerova, A., Ambroz, A., Sram, R., and Hajslova, J.Relationship between atmospheric pollution in the residential area and concentrations of polycyclic aromatic hydrocarbons (PAHs) in human breast milk. *Sci. Total Environ*, **562**: 640–647 (2016).
22. Stefanidou, M., Maravelias, C. and Spiliopoulou, C. Human exposure to endocrine disruptors and breast milk. *Endocr Metab Immune Disord Drug Targets,* **9**(3):269–276 (2009).
23. Perera, F., Tang, D., Whyatt, R., Lederman, S.A.,and Jedrychowski, W. DNA damage from polycyclic aromatic hydrocarbons measured by benzo [a] pyrene-DNA adducts in mothers and newborns from Northern Manhattan, the World Trade Center Area, Poland, and China. *Cancer Epidemiol. Biomarkers Prev,* **14**(3): 709–714 (2005).
24. Hombach-Klonisch, S., Pocar, P., Kietz, S.,and Klonisch, T. Molecular actions of polyhalogenated arylhydrocarbons (PAHs) in female reproduction. *Curr. Med.Chem,* **12**(5):599–616 (2005).
25. Isaac, C. P. J., Sivakumar, A., and Kumar, C. R. P. Lead levels in breast milk, blood plasma and intelligence quotient: a health hazard for women and infants. *Bull Environ Contam Toxicol,* **88**(2):145–149 (2012).
26. Mohd-Taufek, N., Cartwright, D., Davies, M., Hewavitharana, A. K., Koorts, P., Shaw, P. N., Sumner, R., Lee, E., and Whitfield, K. The simultaneous analysis of eight essential trace elements in human milk by ICP-MS. *Food Anal Method,* **9**(7):2068–2075 (2016).
27. Rebelo, F. M.,and Caldas, E. D.Arsenic, lead, mercury and cadmium: Toxicity, levels in breast milk and the risks for breastfed infants. *Environ. Res,* **151**: 671–688 (2016).
28. Leotsinidis, M., Alexopoulos, A.,and Kostopoulou-Farri, E. Toxic and essential trace elements in human milk from Greek lactating women: association with dietary habits and other factors. *Chemosphere,* **61**(2):238–247 (2005).
29. Jacobo-Estrada, T., Santoyo-Sánchez, M.,

Thévenod, F. & Barbier, O. Cadmium Handling, Toxicity and Molecular Targets Involved during Pregnancy: Lessons from Experimental Models. *Int. J. Mol.Sci,* **18**(7):e1590 (2017). https://doi.org/10.3390/ijms18071590.

30. Chao, H. H., Guo, C. H., Huang, C. B., Chen, P. C., Li, H. C., Hsiung, D. Y. and Chou, Y. K.Arsenic, cadmium, lead, and aluminium concentrations in human milk at early stages of lactation. *Pediatr Neonatol,* **55**(2):127–134 (2014).