— **Review** —

Evaluation of Organic Environmental Pollutants Detected in Human Milk

Naoya Kishikawa and Naotaka Kuroda*

Graduate School of Biomedical Sciences, Course of Pharmaceutical Sciences, Nagasaki University, 1–14 Bunkyo-machi, Nagasaki 852–8521, Japan

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Human milk is an important food source for infant because it contains a large number of nutritional substances, growth and immune factors. However, human milk may be contaminated with environmental pollutants when mothers are exposed to these pollutants. In particular, lipophilic organic pollutants are likely to accumulate in milk fat. Therefore, the determination of the organic pollutants levels in human milk is necessary to estimate the health risks of these pollutants to milk-fed infants. For this purpose, a lot of reports for the measurements of environmental pollutants in milk samples have been published. In this review, we summarized the concentrations of harmful organic environmental pollutants such as polychlorinated organic compounds (PCOCs), polybrominated compounds, perfluorinated compounds (PFCs), polycyclic aromatic hydrocarbons (PAHs) and endocrine disrupting phenols in human milk samples. Also, we described the noteworthy results of several evaluative studies such as time trend and regional difference of pollutant levels.

Key words —— human milk, persistent chemical, polycyclic aromatic hydrocarbon, bisphenol A

INTRODUCTION

Human milk is an important source of nutrition for infant because it contains a lot of nutrient substances. Beside the nutrition, the human milk gives some important immune and growth factors to infant to support their healthy life. In these aspects, it is thought that human milk is superior to formula milk for infant feeding. However, if mothers are exposed to harmful environmental pollutants, these pollutants may contaminate human milk and then transfer to infant by milk feeding.[1] In particular, lipophilic organic pollutants are likely to accumulate in human adipose tissue and are excreted in human milk. Therefore, human milk can be considerable contaminated source with lipophilic pollutants for milk-fed infant. Even if the presence of harmful pollutants in human milk is slight, it may give adverse effect on the health of infants who are more sensitive to toxicity of environmental pollutants. Thus, to estimate the infants' risk of exposure to environmental pollutant by lactation, it is important to clarify the amount of these chemicals in human milk. Moreover, the investigation of the concentration of environmental pollutants in human milk is useful to evaluate the contamination degree of lactating women.[2] Until now, the characteristics of pollutants (*i.e.* pollution degree, time trend, regional difference, affecting factor and contamination source) in human milk have been evaluated by a large number of researchers. In this review, we summarized the concentrations of representative organic pollutants such as polychlorinated organic compounds (PCOCs), polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs), polycyclic aromatic hydrocarbons (PAHs) and endocrine disrupting phenols in human milk samples. Additionally, we introduced the noteworthy results of recent studies concerning the evaluation of characteristics of pollutants in milk.

*To whom correspondence should be addressed: Graduate School of Biomedical Sciences, Course of Pharmaceutical Sciences, Nagasaki University, 1–14 Bunkyo-machi, Nagasaki 852–8521, Japan. Tel.: +81-95-819-2894; Fax: +81-95-819-2444; E-mail: n-kuro@nagasaki-u.ac.jp

POLYCHLORINATED ORGANIC COMPOUNDS

PCOCs such as polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) are known as environmental pollutants detected in human milk from a long time ago.[3)] PCBs have been used broadly in electrical equipment such as transformers and capacitors because of their insulation and incombustibility. But in present, production of PCBs is banned and their use is restricted severely due to their high toxicity. PCDFs and PCDDs are generated as unintended by-products during the combustion of organic materials including chlorine in the incineration plants of municipal waste. Additionally, certain organochlorine pesticides (OCPs) including dichlorodiphenyltrichloroethanes (DDTs) or hexachlorocyclohexanes (HCHs) have been detected in human milk even though the agricultural use of OCPs was banned in a lot of countries. Because the PCOCs are extremely persistent, they remain in the environment for a long time. It has been known that these PCOCs have several adverse effects on human health, such as carcinogenicity, immunotoxicity and neurotoxicity, and have lipophilic nature which allows accumulation in human tissues.[4)] The PCOCs are extracted from human milk by non-polar organic solvents such as *n*-hexane and then purified by column chromatography with silica gel, alumina and/or charcoal to separate analytes from co-extractives.[5)] The PCOCs in milk extract are frequently determined by gas-chromatography and mass spectrometry (GC-MS) or gas-chromatography with electron capture detector (GC-ECD).

The concentrations of PCOCs in human milk are often expressed as ng/g lipids basis. Besides, especially in dioxin like chemicals [*i.e.* coplanar PCBs (Co-PCBs), PCDFs and PCDDs], the concentrations are also expressed as toxic equivalent (TEQ) values to estimate total toxicity of a mixture of dioxin like chemicals. The TEQ values is the sum of the concentration of each chemicals multiplied by their specific toxicity equivalence factors (TEF). The results of recent studies on detected concentrations of PCOCs expressed as ng/g and pg-TEQ/g in human milk from different regions in several countries are summarized in Tables 1 and 2, respectively. Generally, the concentrations of PCBs were higher in the developed countries, while the relatively high concentrations of OCPs were detected in developing countries. This indicates that the industrial activities may lead the contamination of PCBs in developed country, whereas the contamination sources of OCPs in developing countries may be derived from their agricultural usage. In some developing country, the use of OCPs had not been prohibited until recently, this may attribute in higher concentrations of OCPs.[6, 7)]

Aballe *et al.* reported that the milk from the mothers in Venice, Italy (industrial area) had PCOCs greater than those from the mothers in Rome, Italy (urban area).[8)] Zhao *et al.* reported that the concentrations of PCBs in milk from the mothers in Luqiao, China (industrial waste disposal area) were higher than those in Pingqiao, China (agricultural area).[9)] These results support that the main possible pollutants source for mother is industrial furniture. However, on the contrary, Schuhmacher *et al.* found that the concentrations of PCBs in milk from mothers living in urban area were higher than those living near industrial area and they suggested that dietary intake is more relevant for human exposure to PCBs.[10)] Likewise, Tajimi *et al.* reported that the PCDFs and PCDDs levels in milk were irrelevant to the distance between the mother's domiciles and the nearest waste incinerators.[11)] Although high concentrations of PCOCs were detected in human milk in some countries, their concentrations in human milk have been decreasing as global trend, because the production and usage of the PCOCs were regulated in a lot of countries and the emission control techniques for waste incinerators have been improved.[12)]

The mother's status can influence the concentration of PCOCs in human milk and thus have been investigated. It is well known that the concentrations of PCOCs in mother increase with age and decrease with the total lactation period. The concentrations of PCOCs were significantly and positively associated with maternal age and it can be attributed to the accumulation of PCOCs in adipose tissues.[13−15)] However, in the case of multipara, the concentrations were not correlated with age.[14, 16)] Because the accumulated PCOCs are eliminated by the excretion in breast milk, the concentrations decrease with the total lactation periods. Inoue *et al.* described that the concentrations of PCBs in milk became lower as the nursing week at milk collection became longer.[17)] The elimination of PCBs through the milk may take part in these results. Nakatani *et al.* found the significant correlation between the concentrations of PCBs in human milk and esti-

Table 1. Mean Concentrations (ng/g lipid) of PCBs, DDTs and HCHs in Human Milk in Different Regions

Survey area	N[a]	Year	PCBs	DDTs	HCHs
Fukuoka, Japan[23]	38	2001–2004	140	340	110
Tokyo, Japan[24]	240	1999–2000	103	—	—
Pingqiao, China[9]	16	2003–2005	208	—	240
Luqiao, China[9]	5	2003–2005	378	—	263
Penang, Malaysia[7]	17	2003	80	1600	230
Jakarta, Indonesia[25]	16	2000	33	630	14
Hochiminh, Vietnam[26]	54	2001	79	2300	14
Chennai, India[6]	12	2002–2003	34	1200	4500
London, United Kingdom[27]	27	2001–2003	204	—	22
Madrid, Spain[28]	11	2004	111	238	—
Duisburg, Germany[16]	169	2000–2003	221	—	—
Athens, Greece[21]	8	2002–2004	94	—	—
Wielkpolska, Poland[22]	22	2004	153	868	14
Buryatia, Russia[17]	17	2003–2004	240	660	810
Arkhangelsk, Russia[13]	23	2002	191	1087	186

a) Sample number.

Table 2. Mean Concentrations (WHO-TEQ pg/g) of Co-PCBs, PCDFs and PCDDs in Human Milk in Different Regions

Survey area	N[a]	Year	Co-PCBs	PCDFs	PCDDs
Fukuoka, Japan[23]	240	2001–2004	10.2	6.3	9.7
Tokyo, Japan[24]	240	1999–2000	11.9	6.8	10.2
Osaka, Japan[18]	48	1999–2000	9.9	6.3	10.2
Tainan, Taiwan[29]	37	2000–2001	—	6.3	8.4
Penang, Malaysia[7]	17	2003	4.5	2.6	5.9
Duisburg, Germany[16]	169	2000–2003	13.4	13.8[b]	
Madrid, Spain[28]	11	2004	3.1	7.8[b]	
Athens, Greece[21]	10	2002–2004	3.2	3.6	3.1

a) Sample number. *b*) PCDFs + PCDDs.

mated seafood consumption before pregnancy.[18] Diversely, Abballe *et al*. observed that there were no relationships between PCOCs levels in milk and fish consumption of mothers.[8] Interestingly, Uehara *et al.* reported that the concentrations of PCDFs and PCDDs in human milk were negatively correlated with smoking habits of the mother.[19] Flesch-Janys *et al.* also found that the blood levels of PCDFs and PCDDs in smokers decayed faster than those in non-smokers and they hypothesized that the cigarette smoking might augment the elimination rate of PCDFs and PCDDs.[20]

The chemicals in human milk are carried through the blood stream. Therefore, the relationships between the concentration of PCOCs in the milk and in the blood from the same donors were studied. Costopoulou *et al.* reported that the concentrations of PCB in the milk were slightly higher than those in the blood.[21] Wittsiepe *et al.* reported that the high-chlorinated PCOCs concentrations in the blood were found in higher than those in the milk, whereas the low-chlorinated PCOCs concentrations in the blood were found in lower than those in milk.[16] These studies revealed that the good correlation between the PCOCs concentrations in blood and milk. Jaraczewska *et al.* observed the weak positive correlation between the PCOCs concentrations in milk and in umbilical cord serum.[22]

POLYBROMINATED DIPHENYL ETHERS

Polybrominated organic compounds, especially PBDEs are also organohalogen compounds frequently detected in human milk samples. PBDEs have been used as flame-retardants to reduce the flammability of plastics and polymers. As similar for PCBs, PBDEs are persistent in the environment and tend to accumulate in adipose tissues. It has been reported that PBDEs causes disruption of thyroid hormone system and decline in learning and memory process in experimental animal models.[30, 31] Therefore, the potential adverse effects resulting from the exposure to PBDEs for infants have become a growing concern. Actually, Chao *et al.* reported that higher levels of PBDEs in human milk were associated with lower birth weight and length.[32] For the measurement of PBDEs in human milk, liquid-liquid extraction, purification by column chromatography and GC-MS or GC-ECD are employed in a similar manner for PCBs because the characteristics of PBDEs are similar to PCBs.[5]

Table 3 summarizes the concentrations of PBDEs in human milk from mothers in several countries. Noteworthy, the concentrations of PBDEs in human milk are significantly higher in Unites States than in other countries. The increasing contamination of human milk with PBDEs may be related to higher usage of PBDEs in United States.[33]

Table 3. Mean Concentrations (ng/g) of PBDEs in Human Milk in Different Countries

Survey area	N[a]	Year	PBDEs
Japan[40]	105	2004	2.5
Taiwan[32]	20	2000–2001	3.9
Indonesia[41]	30	2001–2003	1.3
United States[37]	40	2003	95.6
United States[42]	59	2001–2004	65.9
United Kingdom[27]	54	2001–2003	6.6
Spain[10]	15	2004	2.4
Sweden[39]	13	2001	2.6
Poland[22]	22	2004	2.5
Russia[13]	14	2000	1.2

a) Sample number.

Figure 1 shows a time trend of PBDEs concentrations in human milk from mothers living in Osaka, Japan between 1973 and 2000. In contrast to the decreasing trend of PCBs levels in human milk, the concentrations of PBDEs in human milk have increased continuously since the beginnings of the 1970s when the use of PBDEs have increased.[12, 34–36] However, the concentrations of PBDEs have decreased gradually since the end of 1990s and this may be attributed to the recent development of regulation. She *et al.* observed no positive correlation between the concentration of PBDEs and PCBs in milk samples and suggested that the sources or pathways of human exposure to PBDEs differ from those of PCBs.[37]

The lifestyle characteristics of mothers such as diet consumption and smoking habit, which can affect the concentrations of PBDEs in milk, have been investigated. Ohta *et al.* evaluated the relationship between the concentrations of PBDEs in Japanese human milk and frequency of fish intake of mothers because a higher amount of PBDEs was detected in fish diets as compared to meat or vegetable diets.[38] They obtained a strong relationship between the concentrations of PBDEs in milk and the frequency of fish consumption. Conversely, Lind *et al.* found that the concentrations of PBDEs in milk from Swedish mother were not associated with fish consumption.[39] Lind *et al.* also

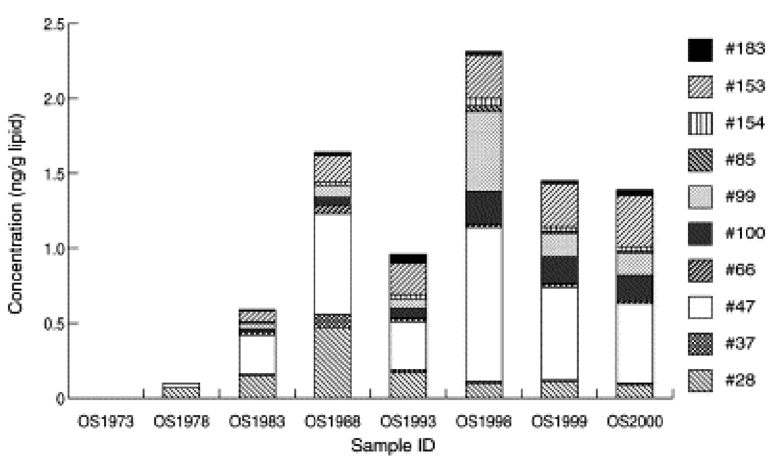

Fig. 1. Time-trend of PBDE Concentrations in Pooled Human Milk Samples Collected from Mothers Living in Osaka between 1973 and 2000

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reported that smoking habit was weakly and positively correlated with the concentration of PBDEs in milk. However, in Japanese study conducted by Eslami *et al.*, no significant difference between smoker and non-smoker was observed for the concentration of PBDEs in milk.[40] These conflicting results may be explained in part by differences of geographical area, food habit and analytical methods.

PERFLUORINATED COMPOUNDS

In recent years, PFCs, notably perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been regarded as toxic chemicals present in human milk. Because PFCs have useful properties such as surface activation ability, water and oil repellent ability and chemical and thermal stability, they have been widely used in a variety of applications such as lubricants, emulsifier, coating agent and fire-fighting foams. The association between PFCs exposure and adverse health effect in human has not been clarified. But, according to the animal experimental results, PFCs cause metabolic and developmental disorders including reduction of birth and postnatal weights.[43] Hence, exposure of PFCs through milk feeding may pose a health hazard for infants. The determination of PFOS and PFOA were carried out by high-performance liquid chromatography (HPLC) with tandem mass spectrometer. For the extraction of PFOS and PFOA from milk, solid phase extraction devices based on anion exchange are frequently employed because PFOS and PFOA have sulfonic acid and carboxylic acid in their structure, respectively.[44–46]

The papers concerning the PFCs levels in human milk are still few. Kärrman *et al.* reported that the mean concentration of PFOS in Swedish milk samples was 0.201 ng/ml, while they could not find a significant amount PFOA in almost of tested human milk samples.[44] They observed the significant positive correlation between the PFOS concentrations in the milk samples and in the corresponding serum samples. But, the concentrations of PFOS in milk were approximately 1% of those in serum. The similar ratio of the PFOS concentration in milk and serum was also observed in the study conducted in United States by Tao *et al.*[45] In this study, the mean concentrations (n = 45) of PFOS and PFOA in human milk were 131 and 43.8 pg/ml, respectively. This study revealed that the concentrations of PFOA in the milk were significant higher for primipara than those for multipara. This result was in agreement with the case of PCBs and suggested that PFOA are excreted in human milk. However, the significant correlation between the concentrations of PFCs and maternal age was not observed, it differed from the result of PCBs. They explained that the difference might be due to the affinity of PFCs to lipoproteins in blood rather than neutral lipids.

So *et al.* reported the concentrations of PFOS and PFOA in Chinese milk samples ranged from 45 to 360 ng/l and from 47 to 210 ng/l, respectively.[46] They also investigated the relationship between the PFCs concentration in milk and diet consumption patterns of mother. Among surveyed diets, only the fish consumption was significant positively correlated with the concentration of long-chained perfluorocarboxylates. Völkel *et al.* determined the concentrations of PFCs in human milk collected in Germany and Hungary, and results were compared.[47] The concentrations of PFOS in milk from Hungarian mother were significantly higher than those from German mother. However, they did not mention the consideration for the results.

POLYCYCLIC AROMATIC HYDROCARBONS

PAHs are unintended products and are originated from the incomplete combustion or pyrolysis of organic matter such as fuel oils. PAHs are widely distributed in the environment due to their release from motor vehicles and various industrial sources.[48] It has been known that a large number of PAHs have carcinogenic and/or mutagenic properties.[49] Though PAHs are largely excreted in urine or feces as hydroxylated metabolites, high membered rings PAHs may be transferred to milk owing to their lipophilicity. Therefore, PAHs in human milk may bring adverse health effects on breast-fed infants.

From these aspects, the determination method for PAHs in human milk was developed,[50] which involves a saponification with sodium hydroxide, liquid-liquid extraction with n-hexane and determination by HPLC with fluorescence detector. By the proposed method, 11 kinds of PAHs could be determined in human milk samples (Fig. 2) and the mean concentrations of total PAHs (Table 4) were found to be 0.75 μg/kg for human milk (n = 51). The concentrations of PAHs in human milk were lower than those in infant formula samples (2.01 μg/kg).

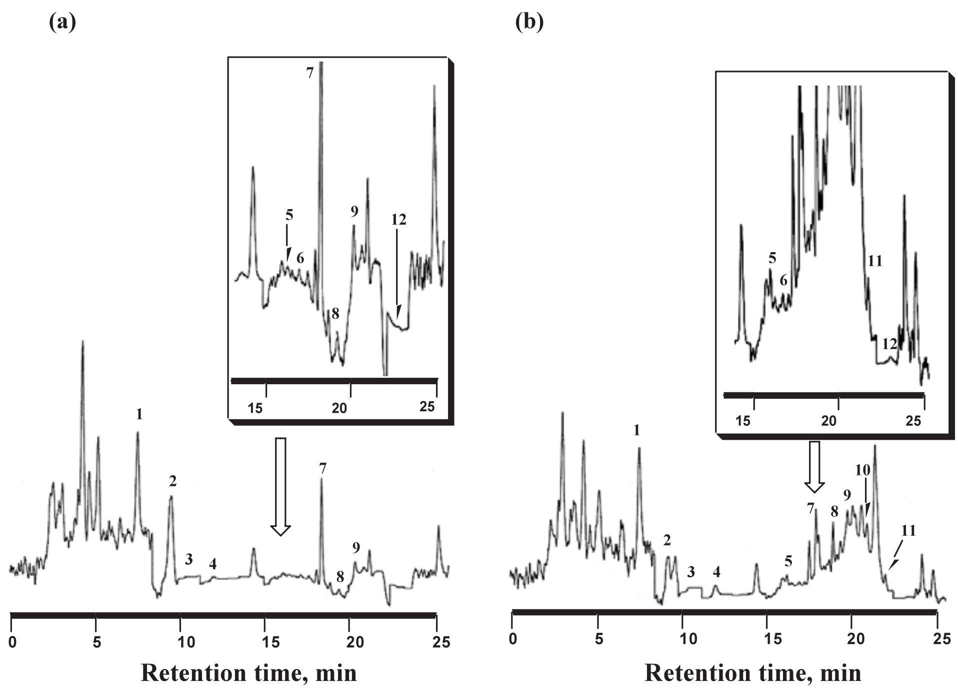

Fig. 2. Chromatograms of PAHs in Human Milk (a) and Infant Formula (b)

Peaks: (1) phenanthrene; (2) anthracene; (3) fluoranthene; (4) pyrene; (5) benzo[*a*]anthracene; (6) chrysene; (7) benzo[*b*]fluoranthene; (8) benzo[*k*]fluoranthene; (9) benzo[*a*]pyrene; (10) dibenzo[*a*,*h*]anthracene; (11) benzo[*g*,*h*,*i*]perylene; (12) indeno[1,2,3-*cd*]pyrene. Reprinted from Ref. 50 with permission of Elsevier Limited.

Table 4. Mean Concentrations (μg/kg) of PAHs in Human Milk and Infant Formula

Compound	Human milk (N = 51)	Infant formula (N = 3)
Phenanthrene	0.25	0.40
Anthracene	0.005	0.02
Fluoranthene	0.02	0.19
Pyrene	0.02	0.16
Benzo[*a*]anthracene	0.004	0.04
Chrysene	0.06	0.25
Benzo[*b*]fluoranthene	0.41	0.36
Benzo[*k*]fluoranthene	0.006	0.05
Benzo[*a*]pyrene	0.002	0.05
Dibenzo[*a*,*h*]anthracene	0.007	0.06
Benzo[*g*,*h*,*i*]perylene	Not detected	0.32
Indeno[1,2,3-*cd*]pyrene	0.003	0.12
Total	0.75	2.01

Furthermore, we observed a positive correlation between the concentrations of PAHs and triglyceride contents in human milk. This result indicates that PAHs are incorporated in milk fat due to their lipophilic nature.

Other than this study, a few studies reported that the concentrations of PAHs in human milk. Kim *et al.* determined 4 kinds of PAHs in human milk from United States women by GC-MS method and the mean concentrations of fluorene, phenanthrene, fluoranthene and pyrene were 0.13, 0.49, 0.06 and 0.05 ng/ml, respectively.[51)] The concentrations of these PAHs are almost comparable with the results in Japanese women. Zanieri *et al.* reported the development of GC-MS method for the determination of PAHs in milk samples and its application to human milk from Italian women.[52,53)] The concentrations of PAHs in milk from Italian mothers are approximately one magnitude higher than those in milk from Japan and United States. As remarkable consequence, they also found that the concentrations of PAHs in milk from smoker and the mother who lived in urban area were significantly higher than those in milk from non-smoker (Fig. 3) and the mother who lived in rural area, respectively. These results suggested that cigarette smoke and polluted air in urban may cause exposure of infants to PAHs through human milk.

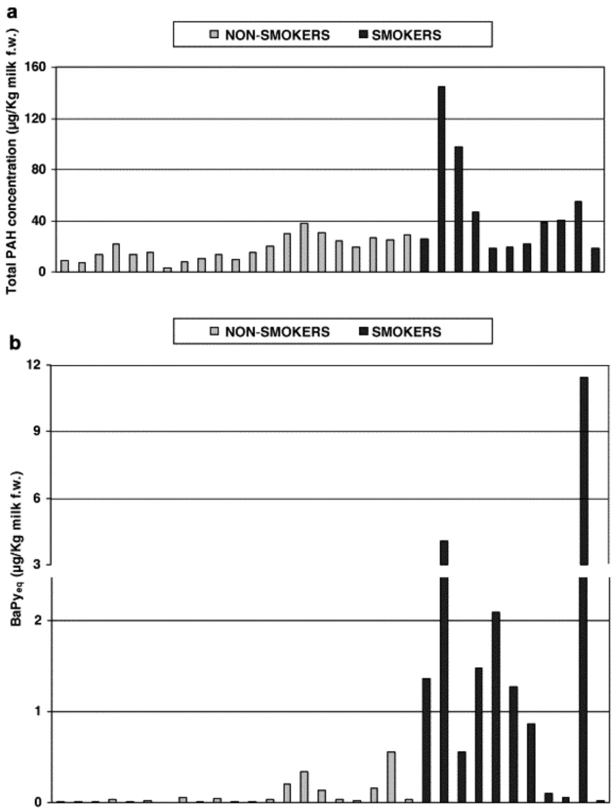

Fig. 3. Total PAH Concentrations (a) and Benzo[*a*]pyrene Equivalent Concentrations (b) Determined in Milk Samples Collected from Non-smokers (n = 21) and Smokers (n = 11)

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ENDOCRINE DISRUPTING PHENOLS

Certain lipophilic phenols have been investigated as possible contaminants in human milk. Bisphenol A (BPA) is an industrial chemical used widely as a raw material of polycarbonate resin. Alkyl phenols such as nonylphenol (NP) and octylphenol (OP) have been used for source materials of industrial, household and commercial applications, especially, non-ionic ethoxylates surfactants. It is known that these phenols exhibit estrogenic activity, which involves the binding to human estrogen receptors, therefore they are referred to as endocrine disrupting chemicals.[54] A sensitive fluorescent method for BPA analysis by using 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoylchloride (DIB-Cl) as a fluorescence labeling reagent was developed, and the method was successfully applied to the determination of BPA in only 0.1 ml of human milk samples.[55] The mean BPA concentration (n = 23) in milk from healthy lactating women was 0.61 ng/ml. But, there was no correlation between the concentration of BPA and triglyceride content. On the other hand, the mean BPA concentration (n = 9) in maternal blood determined by the same method was 0.46 ng/ml and it was the same degree as that in human milk.[56] Kuruto-Niwa *et al.* measured BPA in human colostrums by enzyme-linked immunosorbent assay (ELISA) and found that no significant correlation between the BPA levels and maternal age.[57] Considering these results, BPA may not accumulate in human body even though lipophilic nature. In human body, BPA is conjugated as glucuronide or sulfate and then excreted into urine. Ye *et al.* investigated the concentrations of conjugated and unconjugated BPA in human milk, and reported that the concentrations of total BPA were almost same as those of unconjugated BPA.[58] This observation may be attributed that the conjugated BPA cannot be transported in milk fat due to its low lipophilicity. Because the significant amounts of BPA absorbed in human body remained in blood as conjugated form, there might not be a relationship between the concentrations of BPA in milk and fat content. Ye *et al.* also reported the concentrations of OP in human milk, and mean values were 2.7 ng/ml. Ademollo *et al.* simultaneously determined the concentrations of NP and OP in human milk.[59] The mean concentrations of NP and OP were 16.2 and 0.07 ng/ml, and the fat content of milk was not correlated with both NP and OP levels in milk.

CONCLUSION

Breastfeeding is recommended because it has clear benefits for infants' health and development. But, significant amount of some pollutants has been still detected in human milk even though most of pollutants are in the decreasing tendency. For avoiding the exposure of toxic environmental pollutants to infants, it is necessary to continue the approach to reduce pollution degree in human milk. For example, the appropriate regulation of production and usage of toxic industrial chemicals can contribute for the reduction of pollutants in human milk. Additionally, if the abundant pollutant source or pathway can be clarified, it might be helpful to reduce the pollutant levels in milk. Further surveillance should be required to minimize the concentrations of pollutants in human milk.

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