Congener specific determination of polychlorinated biphenyls (PCBs) in human milk

Abstract

Eighteen congeners Polychlorinated biphenyls (PCBs) congeners were analyzed in 38 human milk samples collected from different ages living in Eastern and Central provinces in Saudi Arabia. The PCB profile was dominated by higher chlorinated congeners. Non-ortho PCB congeners which have the highest Toxic Equivalency Factor (TEF) values were detected in all of individual samples. PCB-81 and PCB-153, the most dominating PCB congeners, which might therefore be used as an indicator for sum PCBs. Toxic Equivalents Factor (TEF) for mono-ortho substituted PCB congeners indicated higher exposure to toxic PCBs in Eastern province rather the Central due to main petroleum industry activities, but estimated daily intakes for both provinces indicate that infants consuming mother’s milk are not at risk of adverse effects caused by PCBs. Our study builds the first database in Saudi Arabia research of human milk samples.

Keywords: saudi Arabia, human milk, PCBs, EDI

Introduction

Many international efforts have been spent to eliminate and/or reduce the emissions and discharges of a wide range of pollutants that have persisted in the environment as well as living organisms. These pollutants are known as a persistent organic pollutants (POPs). Contamination from POPs is a pervasive global problem that urgently demands a global solution. These chemicals include organochlorine (OC) pesticides, such as DDT, endrin, dieldrin, aldrin, chlordane, toxaphene, heptachlor, hexachlorobenzene, mirex. POPs also include industrial chemicals and byproducts, such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofuranes (PCDFs).

PCBs are a mixture of synthetic organic compounds which do not exist naturally in the environment. These compounds are composed of chlorinated biphenyl rings. Theoretically, 209 isomers could be produced, which are different in the degree of chlorination and chlorinated positions. PCBS are hydrophobic, lipophilic, colorless to light yellow, nonflammable, and oily liquids or crystalline solids without smell or taste. Their viscosity, hydrophobility and stability increase as the number of chlorines increases. The half-lives of the congeners range from 1day to 70years. Because of their electrical insulating properties, mixtures of PCBs had been broadly used as coolants and lubricants in electrical transformers, capacitors and hydraulic equipment, and also used as plasticizers in plastic and rubber products. The massive production of PCBs in industrial and commercial applications started in 1929. Following the widespread usage, their hazardous effects on ecosystems and humans were gradually observed, leading to the ban on manufacture in the U.S. in 1977 and the removal of the PCB-containing equipment by 2025 under the Stockholm Convention on Persistent Organic Pollutants.

These chemicals tend to degrade slowly in the environment due to their resistance to biodegradation. Thus, they are prone to biomagnification, in which they exert their toxic effects at different trophic levels, and may also have long half-lives in humans. Certain adverse health and reproductive outcomes have been attributed to these chemicals, including certain cancers, birth defects, and dysfunctional immune and reproductive systems, greater susceptibility to disease and even diminished intelligence. Human milk is the natural and superior food for infants and contains the optimal composition to meet their nutritional needs in early life. Human milk also provides immunological, psychological and economic advantages. Yet, human milk - while still the best food for infants - has been unintentionally compromised by unwelcome chemicals from our environment. The presence of these chemicals is the result of eating, drinking, and living in a technologically advanced world. However, human milk is a unique biological matrix for monitoring certain environmental contaminants because it can provide exposure information about both the mother and breastfed infant through a non-invasive method of collection. Consequently, human milk monitoring can yield information about the types and quantities of POPs in the environment and our bodies. Milk monitoring can also provide a better understanding of our exposure to harmful environmental chemicals, which may help us to better manage such chemicals by eliminating or reducing emissions or limiting their presence in the food supply and environment.

Breast milk monitoring programs have been performed in several countries for investigating geographical and temporal trends in human exposure to POPs. In 2002, the Kingdom of Saudi Arabia signed the Stockholm Convention, and it was ratified in 2012. The present study will be the first comprehensive profile investigating the level of the current PCBs background levels in breast milk of a selected Saudi lactating mother’s population. The aim of this study was to determine individual PCB- congeners, especially the non-ortho substituted PCBs, in Saudi human milk for toxicological assessment. Several non- and mono-ortho PCBs are approximate isomers of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic dioxin congener, and induce similar toxic effects. The levels of the non- and mono-ortho PCBs are given as toxic equivalents (TEQs) in relation to TCDD also included in this study.

Materials and methods

Standards and reagents

PCBs calibration and injection standards were of 99.9% purity and...
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Introduction

PCBs are a class of persistent organic pollutants (POP) that are used in a variety of industrial applications. They are known for their persistence in the environment and their bioaccumulation in food chains. In human milk, PCBs are transferred from the mother to the infant, and they can pose a health risk due to their toxic effects.

Materials and Methods

Sample Collection

Thirty-eight breast milk-feeding women donated about 100mL of breast milk each and collected from five hospitals located in Central and Eastern regions of Saudi Arabia. The samples were collected within the period of 2–8 weeks from their parturition.

Preparation

Each breast milk sample was taken after thawing, homogenized by shaking for 5 minutes before splitting. Each sample was labelled with a unique identification code, 25ml from each sample was taken and diluted 1:1 with phosphate buffered saline and then centrifuged at 2,500 rpm for 10 minutes to isolate the fats and remove cells.

Extraction

Accelerated Solvent Extraction (ASE) 350 from Dionex Inc., Sunnyvale, CA, USA technology was used as extraction techniques and has been shown to produce good recoveries, reliability and sensitivity for PCBs and other POPs in breast milk samples. The ASE process involved the use of a mixture of acetone, dichloromethane and methanol, 3:2:1, v:v:v. Total extraction time was 18 min and total solvent use was 90ml per sample.

Analysis

PCBs analysis was performed using Thermo Scientific™ TSQ 8000™ triple quadrupole GC-MS/MS system equipped with the Thermo Scientific™ TRACER™ 1310 GC Selected reaction monitoring (SRM) 11,000 transition/run. The mode was Electronic Ionization (EI) 70eV, and transition mode for SRM are EI ion source, polarity, positive, electron lens voltage 15V, electron energy 70eV, emission current 50µA, MS transfer line 280°C, Ion source temperature 250°C, and Q1, Q3 frequency 1091.0, 1098.4 respectively. All measurements have been carried out. Injection mode was splitless, Splitless Time 1.0 min, TriPlus RSH Autosampler Injection volume 1µL. GC Column TR™ 5 MS, 30m×0.25mm×0.25µm, Capillary column TR-5MS 30mx0.25mmDx0.25µm for PAHs and PCBs Analysis.

Results

Carrier gas He 99.999%, Flow 1.2 mL/min, constant flow with gas saver unit, Temperature program 100°C, 1 min; 10°C/min to 180°C, 4 min and 10°C/min to 270°C, 2 min, Transfer line temperature 280°C. Total analysis time 46.4 mins, for 33 PCBs congeners. Recovery of analytical method was investigated with 10 spiked milk samples by the 18 congeners at level 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01ng g⁻¹ lipid weight. Recovery of completely analytical method (Table 2) and 10 spiked lipid extracts with three replicates were submitted to the same procedure, described above. Precision of analytical method (repeatability) was also determined, expressed as relative standard deviation, from analyses of 10 spiked samples. Repeatability of GC–MS was estimated by 3 times injection of spiked lipid extract with three replicates (Table 2).

Conclusions

Quality control samples were prepared for each batch of 10 samples as follows: duplicate samples; a blank; a matrix spike sample fortified with the 18 congeners (Table 2) at a concentration of 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01ng g⁻¹ lipid weight. Certified Reference material CRM was prepared for this purpose and processed with each batch of samples. The method limit of detection (LOD) and Limit of Quantification (LOQ), was determined for each compound in the groups State the LOD and LOQ for each compound. Calibration curves were constructed for each PCBs compounds using a series of 10 levels (0.01-10ng/g) of concentrations of each compound that cover the dynamic range in which the targeted compounds are expected to be present. Quality assurance was maintain throughout the course of the study using standard quality assurance procedures and documentation. For external assessment Quality control/Quality assurance, some random analyzed samples sent to reference accredited laboratories were residue-analysis grade 99.9% purity and obtained from Fisher Scientific (Fair Lawn, NJ, USA), Diatomaceous earth (Hidromatrix).

Discussion

The study found that there is a significant level of PCBs in human milk, which poses a health risk to infants. The use of ASE for extraction and GC-MS/MS for analysis provided a reliable and sensitive method for detecting PCBs in human milk. The study also demonstrated the importance of QAQC strategies to ensure the reliability and accuracy of the results.

Conclusion

This study provides valuable information on the levels of PCBs in human milk from Saudi Arabia and highlights the need for further research to understand the potential health risks associated with these pollutants.
lab with the high resolution Gas Chromatography/Mass Spectrometry (HRGC/HRMS).

**Statistical analysis**

Statistical analysis applied to calculate minimum, maximum, mean and standard deviation. All the data subjected for statistical analysis of variance (ANOVA, SPSS, I. (2013). IBM SPSS statistics 22. New York: IBM Corp.) to determine the significance of the categorical factors on different PCBs levels. Any differences caused by variability among the sample groups will be accounted using the SPSS (Statistical Package for the Social Sciences) software (V11 version).

**Research ethics**

The human milk samples collected and the questionnaires completed was used specifically after getting the Ethical Committee approval of each targeted hospitals in central and eastern of Saudi Arabia, for the purposes of this research, and not be employed for other purposes. All provided data in this research project by mothers that may permit their personal identification, and maintained the confidentiality and not be disclosure.

**Results and discussions**

**Demographic features of the participants**

There were 38 participants from the Central and Eastern regions of Saudi Arabia from which 12 from Dammam, 6 from Al-Jubail, 15 from Riyadh and 5 from AL-Kharj. The ages of the participants ranged from 26 to 36years (mean±SD, 31±2.65years). The concentrations of some congener in the human milk samples were < LOD (Table 1) and treated these samples as not detected when calculated the total amount of PCBs.

**Sum of PCBs**

The concentrations of 18 PCB congeners determined in 38 individual samples of human milk are presented in Table 2. Sum PCBs refers to the sum of the mean concentrations of all the congeners listed in Table 2. PCB-81 and PCB-153, the most dominating PCB congeners, which might therefore be used as an indicator for sum PCBs. The obtained results in these surveys seem to be in the middle of the range of data published from other studies and recent published by UNEP10 and11-15 PCB-81 congener was the dominant non-ortho congener (Table 2) (Figure 1). This finding is different from what has been reported in a Swedish and a Canadian human milk survey which showed PCB-126 as the dominating non-ortho congener. The sum of non-ortho PCBs was higher in our study as compared to the Swedish.16 The level of PCB-126 and PCB-169 was higher by 60% and 75%, respectively, in the Norwegian human milk samples as compared to the Swedish human milk. There was a significant differentiation with respect to the location of industrial estates in the area of residence; there were no statistical differences between the age, occupation, and the congener studied.

Different studies done by the WHO to measure the PCBs congeners in human milk in 1987–1988, 1992–1993 and 2001–2003 in several countries,17-19 the levels of contaminants in human milk were lower in the Southern hemisphere (Fiji, Brazil, Philippines, Australia, New Zealand). In the Eastern Europe (Bulgaria, Croatia, Hungary), Ireland and USA, and higher in Western Europe (Italy, Spain, Germany, Luxembourg, Belgium, Netherlands) and in Ukraine, where very high. In addition, Concentrations of PCBs were very high in India (21 and 16.3) but low elsewhere (5.6 and 3.6 in Cambodia, 6.0 and 7.5 in Vietnam, 7.5 and 4.4 in Philippines.20 In Chania, the mean ΣPCB concentration in breast milk from the urban group (n=23) was 42774±27841pg g⁻¹ lipid, which is significantly (p b0.05) higher than in breast milk from the rural group (n=51, 26546±11375pg g⁻¹ lipid.21)

**Risk assessment for infants**

Table 3 showed that the calculated an estimated daily intake (EDI) to examine infants exposure to PCBs compounds. The calculation is based on assumption that a 5kg infant ingests 700g milk per day22 and the following equation was used:

\[
\text{EDI} = \frac{C_{\text{milk}} \times C_{\text{lipid}} \times 700g}{5}
\]

Where EDI is the daily intake (μg/kg body wt./day). Cmilk is the concentration of PCBs in the milk (μg/kg lipid wt.) and Clipid is the lipid content in milk (%). The results are given in Table 3 as ranges and medians of individual intakes. Calculated EDI values are one to two orders of magnitude lower than TDI, except for PCBs (the highest individual EDI is two times lower than corresponding TDI). Based on these results we can say with certainty that infants consuming human milk analyzed in this study are not at risk of adverse effects caused by organochlorine compounds (Table 3).

| No | IUPAC # | CAS # | Structure | TEF | LOD (μg/kg) | Recovery % |
|----|---------|-------|-----------|-----|-------------|------------|
|    |         |       |           |     | WHO 2005    |            |
|    |         |       |           |     | ± SD        |            |
| 1  | 28      | 7012-37-5 | 2,4,4'-Trichlorobiphenyl | NA  | 0.005       | 100.77±2.08 |
| 2  | 52      | 35693-99-3 | 2,2,5,5'-Tetrachlorobiphenyl | NA  | 0.004       | 97.89±3.22  |
| 3  | 77      | 32598-13-3 | 3,3',4,4'-Tetrachlorobiphenyl | 0.0001 | 0.005     | 98.84±2.66  |
| 4  | 81      | 70362-50-4 | 3,4,4',5'-Tetrachlorobiphenyl | 0.0003 | 0.004     | 99.39±4.33  |
| 5  | 101     | 37680-73-2 | 2,2,4,5,5'-Pentachlorobiphenyl | NA  | 0.005       | 98.42±2.51  |
| 6  | 105     | 32598-14-4 | 2,3',4,4',5'-Pentachlorobiphenyl | NA  | 0.005       | 98.64±2.74  |
| 7  | 114     | 74472-37-0 | 2,3,4,4',5'-Pentachlorobiphenyl | 0.00003 | 0.003     | 101.59±2.49  |
| 8  | 118     | 31508-00-6 | 2,3',4,4',5'-Pentachlorobiphenyl | 0.00003 | 0.004     | 102.09±2.88  |
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Table 2 Statistical parameters for most contributed PCBs congeners (ng/kg) to ΣPCBs in mother milk

| No | IUPAC # | CAS #   | Structure                       | TEF       | LOD (µg/kg) | Recovery % |
|----|---------|---------|---------------------------------|-----------|-------------|------------|
|    |         |         |                                 | WHO 2005  | ± SD        |            |
| 9  | 123     | 65510-44-3 | 2',3,4,4',5'-Pentachlorobiphenyl | 0.00003   | 0.005       | 99.6±2.80  |
| 10 | 126     | 57465-28-8 | 3',3',4,4',5-Pentachlorobiphenyl | 0.1       | 0.005       | 102.43±1.75 |
| 11 | 138     | 35065-28-2 | 2',2,3,4,4,5'-Hexachlorobiphenyl | NA        | 0.005       | 96.71±1.77  |
| 12 | 153     | 35065-27-1 | 2,2,4,4,5,5'-Hexachlorobiphenyl | NA        | 0.005       | 103.73±2.61 |
| 13 | 156     | 38380-08-4 | 2,3',4,4',5'-Hexachlorobiphenyls | 0.00003   | 0.003       | 99.82±3.04  |
| 14 | 157     | 69782-90-7 | 2,3,3',4,4',5'-Hexachlorobiphenyl | 0.00003   | 0.003       | 98.84±2.41  |
| 15 | 167     | 52663-72-6 | 2,3',4,4',5,5'-Hexachlorobiphenyl | 0.00003   | 0.003       | 99.05±2.18  |
| 16 | 169     | 3274-16-6  | 2,2,3,4,4,5,5'-Heptachlorobiphenyl | 0.03      | 0.003       | 97.43±1.44  |
| 17 | 180     | 35065-29-3 | 2,2,3,4,5,5,5'-Heptachlorobiphenyl | 0.0001    | 0.005       | 95.5±3.11   |
| 18 | 189     | 52712-05-7 | 2,3',4,4',5,5'-Heptachlorobiphenyl | 0.00003   | 0.005       | 98.75±2.55  |

Table Continued.

Statistical parameters for most contributed PCBs congeners (ng/kg) to ΣPCBs in mother milk

| PCB-77 | PCB-81 | PCB-126 | PCB-169 | PCB-105 | PCB-114 | PCB-118 | PCB-123 | PCB-156 | PCB-157 | PCB-167 | PCB-189 | PCB-28 | PCB-52 | PCB-101 | PCB-138 | PCB-153 | PCB-180 |
|---------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|--------|--------|---------|---------|---------|---------|
| ΣPCBs   | 34.71  | 94.02   | 6.38    | 30.98   | 26.6    | 21.76   | 39.71   | 5.26    | 5.67    | 5.77    | 1.36    | 35.7   | 14.13  | 4.86    | 2.83    | 52.34   | 62.25   | 44.65   |
| mean    | 1.93   | 5.22    | 0.38    | 1.72    | 1.48    | 1.21    | 2.21    | 0.29    | 0.33    | 0.36    | 0.1    | 1.98   | 0.79   | 0.24    | 0.22    | 2.91    | 3.46    | 2.48    |
| median  | 1.8    | 0.89    | 0.32    | 1.38    | 1.35    | 1.2     | 2.03    | 0.3     | 0.31    | 0.32    | 0.08   | 1.86   | 0.81   | 0.19    | 0.07    | 2.57    | 3.46    | 2.31    |
| Max     | 3.31   | 7.7     | 0.78    | 3.27    | 2.31    | 2.82    | 3.88    | 0.73    | 0.77    | 0.78    | 0.34   | 3.31   | 1.55   | 0.48    | 0.72    | 5.31    | 5.12    | 3.51    |
| Min     | 0.94   | 0.35    | 0.18    | 1.04    | 1.01    | 0.14    | 1.25    | 0.07    | 0.13    | 0.18    | 0.04   | 0.94   | 0.26   | 0.12    | 0.04    | 0.69    | 0.92    | 1.58    |
| SD      | 0.77   | 17.92   | 0.18    | 0.64    | 0.43    | 0.77    | 0.75    | 0.15    | 0.17    | 0.16    | 0.08   | 0.79   | 0.36   | 0.12    | 0.24    | 1.25    | 1.15    | 0.59    |
| ΣPCBs   | 34.89  | 52.42   | 3.25    | 24.62   | 23.38   | 30.84   | 28.82   | 4.27    | 4.18    | 3.4     | 4.24   | 34.89  | 7.01   | 3.52    | 4.05    | 44.91   | 40.24   | 38.21   |
| Mean    | 1.71   | 2.81    | 0.16    | 1.29    | 1.17    | 1.78    | 1.42    | 0.22    | 0.22    | 0.17    | 0.22   | 1.71   | 0.36   | 0.21    | 0.21    | 2.11    | 2.02    | 1.78    |
| median  | 1.53   | 0.65    | 0.09    | 0.99    | 1.05    | 1.33    | 1.26    | 0.08    | 0.08    | 0.08    | 0.16   | 1.53   | 0.32   | 0.14    | 0.09    | 2.03    | 1.83    | 1.66    |
| Max     | 4.2    | 3.7     | 0.58    | 3.25    | 2.22    | 3.27    | 2.44    | 0.78    | 0.77    | 0.78    | 0.7    | 4.2    | 0.78   | 0.55    | 0.79    | 4.42    | 3.52    | 3.43    |
| Min     | 0.98   | 0.24    | 0.03    | 0.57    | 0.52    | 1.04    | 0.88    | 0.04    | 0.04    | 0.03    | 0.05   | 0.98   | 0.18   | 0.08    | 0.03    | 1.36    | 0.94    | 0.91    |
| SD      | 0.76   | 8.11    | 0.17    | 0.66    | 0.53    | 0.69    | 0.49    | 0.25    | 0.25    | 0.22    | 0.2    | 0.76   | 0.17   | 0.15    | 0.26    | 0.88    | 0.83    | 0.85    |
**Table 3** Range (median) of estimated daily intake (ng/kg body wt./day)

| Eastern provence | Central provence | EDI   |
|------------------|------------------|-------|
| 0.298–0.053 (0.086) | 0.178–0.035 (0.065) | 1.0 μg/kg body wt./day |

**Conclusions**

The results of this study have provided a baseline level for 18 PCBs congeners in the first study investigating the PCBs levels in mother breast milk in eastern and central of Saudi Arabia. The estimated EDI of breast-fed infants were lower those of some non-exposure areas in central and eastern of Saudi Arabia and that infants consuming human milk analyzed in this study are not at risk of adverse effects caused by tested PCBs, campaign to the exposure levels of many developed countries. The level of PCBs in eastern were higher than central areas may be due to the industrial activates in eastern areas. This will greatly help the Saudi government take steps to control and eliminate these POPs in the future.

**Acknowledgements**

This Project was funded by the National Plan for Science, Technology & Innovation (MAARIFAH), King Abdulaziz City for Science & Tech. Kingdom of Saudi Arabia, Award and Project No. 12-ENV-2585-2.

**Conflict of interest**

The author declares no conflict of interest.

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