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Application of Kohonen neural network for evaluation of the contamination of Brazilian breast milk with polychlorinated biphenyls

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ABSTRACT

Due to the tendency of polychlorinated biphenyls (PCB) to accumulate in matrixes with high lipid content, the contamination of the breast milk with these compounds is a serious issue, mainly to the newborn. In this study, milk samples were collected from breastfeeding mothers belonging to 4 Brazilian regions (south, southeast, northeast and north). Twelve PCB were analyzed by HS-SPME-GC-ECD and the corresponding peak areas were correlated to the answers to a questionnaire of general habits, breastfeeding and characteristics of the living places. To realize this exploratory analyze, self-organizing maps generated applying Kohonen neural network were applied. It was possible to verify the occurrence of different PCB congeners in the breast milk relating to the region of the Brazil that the breastfeeding lives, the proximity to an industry, the proximity to a contaminated river or sea, the type of milk (colostrum, foremilk and hindmilk) and the number of past pregnancies.

1. Introduction

Polychlorinated biphenyls (PCB) are an important class of widespread contaminants due to their high persistence in the environment and to their tendency to accumulate on soil, plants and animals and to propagate in the trophic level of food chain [1,2]. People are exposed to PCB primarily from the dietary intake (ca. 90% of the cases) [3], as well as by the contaminated atmosphere.

Children are exposed to PCB in the same way as adults but because of their smaller weight, the corresponding intake PCB per kilogram of body weight may be greater. In the case of fetuses and toddlers, the transference from mother happens via placenta blood exchange and breast milk, respectively [4], and poses a serious risk to health, since their enzymatic and metabolic systems are not yet mature [5]. The PCB concentration in breast milk is higher when compared with maternal and cord serum [6], and therefore it is the preferred matrix to evaluate human background exposure [7,8].

Taylor et al. [9] compared some data obtained from women that work in capacitor manufacturing facilities (a typical environment where PCB exposition may be high) with women that work in lower PCB exposition area. The babies whose mothers work in the first situation weighed significantly less at birth than the babies from the other mothers. Also, this high exposure to PCB was also associated with shortened pregnancy period. A survey of 128 children known to have been in uterus during or after Yu-Cheng exposure found that mean birth weight was decreased by approximately 15% compared to a control group of 115 non-exposed children [10].

Other studies concluded that neurotoxic effects of PCB and dioxins in prenatal exposure may persist into school age and may result in cognitive and motor developmental subtle delays [11,12]. Rylander et al. [13] related that the consumption of contaminated fish by girls during infancy and adolescence increases their risk of generating below-average weight babies. Some studies suggest as well that the immune system may be affected in children born and nursed by mothers exposed to increased levels of PCB [14].

In response to their adverse effects, the production and use of PCBs were restricted and banned in Europe and North America during 1970s; in Brazil, they were prohibited only in 1981 [15].

Among the principal factors that lead to the high levels of PCB in human breast milk, the most important are the lipid content of this matrix, the time delayed from the beginning of nursing, the age, the weight and number of previous pregnancies of the mother, her origin, her living place, her alimentary and smoking habits, as well as seasonal and occupational factors [2,16]. Vaclawik et al. [17] showed that age, body mass index (BMI), lactation and the consumption of fish with high fat content were all

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consistently associated with high PCB levels in adipose tissue on Danish women. LaKind et al. [18] concluded that the levels in women today can be attributed principally to accumulation during lifetime, so there are no immediate actions that women can take to reduce the exposure of their toddlers during breastfeeding breast-fed infants' exposure.

Information regarding women lifestyle can be correlated to analytical data to monitor and point out possible sources of PCB contamination. However, the evaluation of such large number of possible influencing variables in the PCB content in breast milk can be difficult or even impossible without proper data treatment tools. Chemometric resources such as self-organizing maps (SOM) generated through Kohonen neural network [19] can be appropriate to these exploratory analysis, by the possibility of evaluating distribution of samples and influence of variables on a bi-dimensional graphics (maps) [20,21].

In this paper, twelve PCB in breast milk from 193 voluntary donating lactating mothers from 10 cities and towns spread through Brazil were detected, identified and quantified by headspace solid-phase microextraction (HS-SPME) combined to gas chromatography with electron capture detection (GC-ECD), and the resulting data matched to general habits, breastfeeding and environmental conditions of the living place using maps generated by Kohonen neural networks.

2. Experimental

2.1. Reagents and material

Twelve PCB standards (IUPAC numbers 28, 52, 74, 101, 118, 128, 138, 153, 156, 170, 180 and 187) were obtained from AccuStandard (New Haven, CT, USA). Methanol was obtained from Merck (Darmstadt, Germany), reagent grade NaCl from Ecibra (São Paulo, Brazil) and pesticide grade isooctane from Mallinckrodt (Kentucky, USA). Deionized water was purified through a Milli-Q system (Millipore, Bedford, MA, USA). Helium (99.999% purity) and nitrogen (99.999%) were supplied by White Martins (Rio de Janeiro, Brazil).

SPME fiber coated with 100 μm polydimethylsiloxane (PDMS) fiber were supplied by Supelco (Bellefonte, PA, USA) and fit in the appropriate holders (Supelco). Septum-sealed 16 mL glass vials were obtained from Pierce (Rockford, IL, USA). All glassware were silanized with a 10% solution of chlorotrimethylsilane in toluene as described by Potter et al. [22].

2.2. Gas chromatograph

All analyzes were performed on an AutoSystemXL GC-ECD system (Perkin-Elmer, Norwalk, CT) fitted with a HP-1MS column (30 m × 0.32 mm × 0.25 μm) and ^{63}Ni electron-capture detector. The split-splitless injector was operated in splitless mode and fitted with a suitable liner for SPME. Injector and detector temperatures were set to 280 °C and 320 °C, respectively. Helium was used as carrier gas with a flow rate of 1.3 mL/min and nitrogen was used as detector make-up gas. The column oven temperature was programmed as follows: 40 °C for 2 min, then 30 °C/min to 190 °C, hold for 5 min, then 5 °C/min to 220 °C, hold for 5 min, 20 °C/min up to 300 °C and hold for 1 min.

2.3. SPME procedure

The samples were kept frozen until use. To a 5.00 mL aliquot of sample, 1.80 g of NaCl and 210 μL of methanol were added; the sample suspension was magnetically stirred at 1200 rpm for 10 min to sample/headspace equilibration and then a 100 μm PDMS SPME fiber exposed to the sample headspace for 60 min. During sample/headspace equilibration and extraction, the vial was kept at 95 °C. All the extraction conditions were performed as defined in previous studies [23]. Immediately after extraction the fiber was withdrawn and the analytes immediately desorbed directly in the GC-ECD injection port at 280 °C for 5 min.

2.4. Study subjects

A cohort of 193 breast-feeding women from different parts of Brazil was evaluated, according to Fig. 1. The samples (mean of 20 mL) were frozen immediately after collection and kept at −20 °C until the analysis be performed.

The National Commission on Ethics in Research (CONEP) and the Ethics Committee of the Local Health Units approved the study protocol. In each Mother Milk Bank a nurse was orientated to explain and obtain informed consent from each voluntary allowing the use of samples of their milk on this work. Short questionnaires were also applied to each mother including questions related to personal features and habits, lifestyle, economic and social status, occupation and residence history, consume of water on the immediate period before breastfeeding and type of milk produced at that moment (colostrum, transition milk or mature milk).

2.5. Kohonen neural network processing

All calculations were performed using MATLAB 7.9 programming environment (the MathWorks, Natick, MA) and the public domain SOM toolbox [24].

Samples were organized according their city of origin and an exploratory analysis was performed for each resulting group in order to point out samples with a profiles. This preliminary proccessing was used to reduce the size of the data set (193 samples), selecting only samples representative the data variability; it was found the 50 samples that could be used to represent the whole original data set.

Fig. 1. Brazilian map: 1 – Florianópolis/Santa Catarina; 2 – Curitiba/Paraná; 3 - Santo Amaro/São Paulo; 4 – Belenzinho/São Paulo; 5 – Vitória/Espírito Santo; 6 – Araxá/Minas Gerais; 7 – Aracajú/Sergipe; 8 – Natal/Rio Grande do Norte; 9 – São Luis/Maranhão and 10 – Rio Branco/Acre.