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Article

Determination of Polychlorinated Biphenyls in Brazilian Breast Milk Samples using Solid-Phase Microextraction and Gas Chromatography-Electron Capture Detection

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Um método para a determinação de bifenilas policloradas (PCB) no leite materno foi desenvolvido e aplicado para avaliar a contaminação de amostras procedentes de quatro cidades brasileiras. Os PCB foram extraídos através da Microextração de Fase Sólida e analisados por Cromatografia Gasosa com Detector por Captura de Elétrons. As figuras de mérito estudadas foram linearidade (1 a 16 μg L^{-1}, $r > 0{,}9884$), precisão (RSD < 12%, n = 5), recuperação (71 a 127%) e limite de quantificação (entre 0,45 e 2,42 μg L^{-1}). A análise das vinte amostras revelou níveis de PCB acima de 11,8 μg L^{-1} na região metropolitana de São Paulo, sendo o PCB 153 encontrado em todas essas amostras. Em Vitória/ES e Florianópolis/SC foram encontrados PCB em 100 e 60% das amostras, respectivamente. Nenhuma contaminação foi detectada nas amostras do Rio Branco/Acre. Sendo assim, uma alta correlação entre a contaminação dessas amostras e o nível de industrialização da região foi encontrada.

A method for the determination of polychlorinated biphenyls (PCB) in breast milk was developed and applied to evaluate the contamination of samples that proceed from four Brazilian cities. PCB were extracted by Solid-Phase Microextraction and analyzed by Gas Chromatography with Electron Capture Detector. The figures of merit studied were linearity (to 16 μg L^{-1}, $r > 0.9884$), precision (RSD < 12%, n = 5), recovery (71 to 127%) and limit of quantification (between 0.45 and 2.42 μg L^{-1}). The analysis of the twenty samples revealed PCB levels above 11.8 μg L^{-1} in São Paulo metropolitan area being the PCB 153 found in all these samples. In Vitória/ES and Florianopolis/SC were found PCB in 100 and 60% of the samples, respectively. No contamination was detected in the samples from Rio Branco/AC. Thus, a high correlation between the contamination of these samples and the level of industrialization of the region was found.

Keywords: PCB, HS-SPME, validation, breast milk, contamination

Introduction

Polychlorinated biphenyls (PCB) have been used commercially since 1929 as dielectric and heat exchange fluids as well as in a variety of other applications. However, the distribution of PCB in the environment was not recognized until 1966, when Jensen identified PCB in human and wildlife samples.[1] Owing to their physicochemical properties, these compounds are extremely resistant to chemical and biological degradation and are easily bioaccumulated through the trophic pyramid,[2,3] especially due to their lipophilic character (which causes their accumulation on fat tissues).[4,5] Therefore, the PCB contamination is a serious issue, and despite the fact that their production and use has been banned during the 1970's and 1980's, they are still detected in the ecosystem.[6-8] In Brazil, a law implemented in 1981 prohibits the importation, the manufacture, the use and the marketing of PCB and demands the substitution of the electric system equipments in operation only for others that do not contain PCB. Besides, the law prohibits to discard these equipments in sanitary landfills and courses of water.

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The primary immediate intake route of organochlorine pesticides and PCB congeners for the general population has been identified as being food-related, mainly due to the consumption of contaminated dairy products, meat and fish.[9-12] It is now well known that PCB can be transferred from mother to fetus through the placenta, as well as to newborn babies being fed with breast milk.[13] These infants will probably have higher risks to develop immunosuppression, neuropathy, liver damage and cancer.[14]

Due to its high concentration levels usually found in breast milk, and since it is comparably easier to collect than other alternatives, breast milk has also been employed in studies of human background exposure to PCB.[15,16] The most abundant PCB congeners found in breast milk are PCB **153**, **180**, **138**, **170**, **118**, **101** and **187** (referred as their IUPAC numbers).[17-21] All these PCB, except PCB **118**, are non-coplanar and ortho-substituted, and classified as non-dioxin-like species. The effects of non-dioxin-like PCB are related to multiple toxicity pathways, even though sometimes they have similar action to their dioxin-like counterparts.[22] Regulatory limits for PCB contamination in foodstuff vary from 20 μg Kg^{-1} to 60 μg Kg^{-1},[23] depending on the country. As for the situation in Brazil, there is no specific legislation regarding the maximum equivalent concentration levels of organochlorine compounds in breast milk;[24] besides, the degree and level of contamination are not well known. One of the few studies was performed by the National Agency for Sanitary Surveillance,[25] and showed that the levels of dioxin-like PCB (the most toxic) in breast milk collected in Brazil varied from 1.30 to 12.30 pg (PCB) g^{-1} of milk fat.

The conventional analytical procedures for the determination of PCB and similar species on complex matrices involve preliminary steps, such as their isolation by liquid-liquid extraction (LLE), combined with the clean-up and purification of extracts to remove impurity before the chromatographic separation.[26] A relatively large amount of sample is required and besides, it is necessary to use large amounts of toxic organic solvents. In 1990, Arthur and Pawliszyn[27] introduced the Solid Phase Microextraction (SPME), a solvent-free sample preparation technique, to replace such conventional procedures, which has been used extensively to a wide variety of samples,[28] including the determination of PCB in some matrices.[29,30] SPME is based on the sorption of analytes in samples or on its headspace by a film of up to 100 μm of an extracting phase (either pure liquid polymeric phases or porous solids dispersed in liquid polymers) over a silica fiber. For liquid polymeric phases such as polydimethylsiloxane (PDMS), partition is the mechanism involved; for porous solid coatings (Carboxen/PDMS), there is a mix of partition and adsorption, with large predominance of the latter. When the separation and detection of analytes are performed by Gas Chromatography (GC), after introduction of the fiber directly inside the injection port of the chromatograph, the extracts can be thermally desorbed. Since the kinetics and thermodynamics of the extraction are governed by experimental conditions such as temperature, time, ionic strength of the sample, stirring rate, headspace volume and addition of co-solvents, the optimization of extraction parameters is fundamental. The optimization of SPME methods can be performed by using either classic unvaried approaches or, more conveniently, multivariate procedures such as the factorial design.[31] For the separation and detection of PCB, the use of GC coupled with Electron Capture Detection (ECD) is almost universal.[32,33] Despite its high sensibility for halogenated contaminants, ECD neither is specific nor provides qualitative information, and the PCB identification should be confirmed by retention data combined to GC-MS.[34,35]

In a previous paper,[31] a headspace SPME method combined with GC-ECD to determine PCB in breast milk was optimized by using a chemometric multi-optimization procedure. For that, an experimental design arranged according to Doehlert matrixes was used to generate the necessary data. Artificial neural networks were employed to find a model that correlates the PCB peak areas with SPME operational parameters. Finally, genetic algorithm was applied to this model to find the operational conditions that provides the maximized chromatographic responses for all evaluated analytes simultaneously. Therefore, in this study we present the validation of this method, which was also extended to GC-MS and finally applied to identify and quantify PCB in breast milk samples collected from different Brazilian regions.

Experimental

Reagents and materials

Standards of twelve PCB (IUPAC # **28**, **52**, **74**, **101**, **118**, **128**, **138**, **153**, **156**, **170**, **180** and **187**) were purchased from AccuStandard (New Haven, CT, USA). Methanol was obtained from Merck (Darmstadt, Germany), NaCl P.A. from Ecibra (São Paulo, Brazil) and isooctane (pesticide grade) 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 fibers coated with 100 μm (PDMS) were supplied by Supelco (Bellefonte, PA, USA); the fibers

were fit in an appropriate holder (Supelco). Septum-sealed 16 mL glasses were obtained from Pierce (Rockford, IL, USA). All glassware was silanized with a 10% solution of chlorotrimethylsilane in toluene as described by Potter.[36] For cleaning and decontamination of the glassware and the magnetic stir bars prior to use, they were washed with neutral detergent and deionized water and boiled with deionized water for 10 minutes in a microwave oven, followed by rinsing with deionized water and dried in oven at 70 °C. During the extractions, the samples were thermostated by using a heated circulating bath (Cole Parmer, USA).

Instrumentation

The GC-ECD system was an AutoSystemXL GC-ECD (Perkin-Elmer, Norwalk, CT) fitted with a HP-1MS column (30 m ×0.32 mm × 0.25 μm) and a ^{63}Ni electron-capture detector. The split-splitless injector was operated in the splitless mode and fitted with a suitable liner for SPME. The injector and detector temperatures were 280 °C and 320 °C, respectively. Helium was used as a carrier gas at a flow rate of 1.3 mL min^{-1} and nitrogen was the make-up gas. Shimadzu 17A GC was the GC-MS system used to confirm the results, coupled with a Shimadzu QP-5000 quadrupole mass spectrometer (Shimadzu, Tokyo, Japan) operated by using full scan and selective ion monitoring mode (SIM). In this case, the separation was carried out by using a DB-5 (30 m × 0.25 mm × 0.25 μm) and both injector and interface temperatures were 280 °C. Helium was used as a carrier gas at a flow rate of 1.3 mL min^{-1}. Full-scan mass spectra were obtained in the electron ionization mode (70 eV) in the range from *m/z* 100 to 400 for analyte identification. On SIM runs, the target and secondary ions monitored for each PCB were (IUPAC congener number and fragment *m/z*): PCB **28** (186, 256 and 258), PCB **52** and **74** (220 and 292), PCB **101** and **118** (254 and 326), PCB **153**, **138**, **128** and **156** (290 and 360) and PCB **187**, **180** and **170** (324 and 394). In both chromatographic systems, the oven temperature was programmed as follows: 40 ºC (hold for 2 min), then 30 ºC min^{-1} to 190 ºC (hold for 5 min), then 5 ºC min^{-1} to 220 ºC (hold for 5 min) and 220 ºC min^{-1} up to 300 ºC (hold for 1 min).

Sampling and storage

This study analyzed 20 breast milk samples collected between 2005 and 2006 from Human Milk Banks (five samples per bank) associated to public health services in the metropolitan areas with diversified profiles: São Paulo (capital of the state of São Paulo, population 10.9 million, heavily industrialized and highly polluted), Vitória (capital of the state of Espírito Santo, pop. 0.31 million, export harbor), Florianopolis (capital of the state of Santa Catarina, pop. 0.4 million, mainly leisure and tourist activity) and Rio Branco (capital of the state of Acre, pop. 0.29 million, urban center surrounded by the Amazon rainforest). It should be noticed that sample donators are not necessarily residents in the collection area, since mothers from bordering regions not serviced by public health facilities usually need to travel to these larger centers for assistance. After collection, they were immediately frozen and stored at –20 ºC until the analysis. The procedure was previously examined and approved by the National Commission of Research Ethics (CONEP), as well as by the local Ethics Committees from the public health offices involved.

SPME procedure

For all extractions, the samples were defrosted and 5.00 mL immediately transferred to 16 mL silanized glass vials. To increase the extracted masses, 1.8 g of NaCl and 210 μL of methanol were added in the media. These additions are well established in the SPME theory: the presence of electrolytes, such as NaCl in aqueous samples decreases the activity of the solvent and increases the activity of the analyte (specially for non-polar analytes); besides, the addition of small amounts of water-miscible organic solvents, such as methanol or acetonitrile, can improve the extraction efficiency of highly hydrophobic species (such as the PCB), due to the increase on the solubility of the analytes in the media. The samples were magnetically stirred at 1200 rpm for 10 min to permit sample/headspace equilibration. A 100 μm PDMS SPME fiber was exposed to the sample headspace for 60 min of time. During sample/headspace equilibration and extraction, the sample was thermostated at 95 °C. After extraction the fiber was withdrawn and extracted analytes were immediately desorbed directly in the GC-ECD or GC-MS injection port at 280 °C for 5 min. To minimize inter-extractions fiber carryover, blank runs were performed between each extraction.

Validation and application to real samples

For all validation experiments, pooled blank breast milk was prepared from samples found to be non-contaminated in preliminary assays. The blank sample was spiked with 0.5 μg L^{-1} to 64 μg L^{-1} of each PCB evaluated. The spiked samples were kept at 4 °C for 24 h to allow the equilibration between the PCB and the lipid fraction and other possible active biological milk compounds.[37] This sample set

was employed to determine the figures of merit of the HS-SPME-GC-ECD method. Linearity was evaluated through the calibration curve in eight different levels (0.5, 1, 2, 4, 8, 16, 32 and 64 μg L^{-1}) and thus the linear range was obtained. To calculate the LOD and LOQ of each compound, the standard deviation of the linear coefficient (σ) divided by the slope or angular coefficient (S) obtained from the calibration plot was considered. In both cases, the result was multiplied by 3.3 and 10.0, respectively.[38,39] The precision was estimated through the repeatability obtained by five successive extractions of samples spiked with 4 μg L^{-1} of each PCB being the result expressed as relative standard deviation (% RSD). The accuracy was evaluated at the same five levels on which the calibration curve was performed. Each point was subtracted, the equation was recalculated and the theoretical area was compared with the experimental area and reported as percent recovery. The selectivity of the method was assessed by inspecting GC-MS chromatograms for the test samples. Finally, the validated method was applied to the 20 breast milk samples.

Results and Discussion

Method evaluation

It is well known that SPME is an extraction technique affected by the matrix and this fact needs to be considered mainly in trace analysis. Therefore quantitative measurements in real samples normally require applying the standard addition technique.[40] So, to perform all the validation steps, a non contaminated pooled milk was spiked with the mixture of PCB congeners. The Figure 1A shows a blank milk sample chromatogram and the Figure 1B shows a chromatogram obtained by this blank milk spiked with 9.25 μg L^{-1} of each PCB. Through Figure 1B, it was observed that the peak resolution was adequate for the target analytes identification and quantification.

The figures of merit are shown in Table 1. The linearity of the proposed method was evaluated from 0.5 to 64 μg L^{-1} of each compound however, a linear correlation between amount and concentration was found between 0.5 and 16 μg L^{-1} (PCB **128**, **156**, **170** and **180**) and between 1 and 16 μg L^{-1} to the others. This range is in agreement with other studies since the real concentration of these compounds in the majority of breast milk analyzed around the world is allocated within this range. Calibration curves (peak area *versus* concentration) were obtained for the 12 PCB, being all correlation coefficients (r) higher than 0.9884.

LOD values ranged from 0.45 μg L^{-1} (PCB **180**) to 2.42 μg L^{-1} (PCB **74**) and LOQ values ranged from 1.37 μg L^{-1} to 7.34 μg L^{-1} to the same PCB (Table 1).

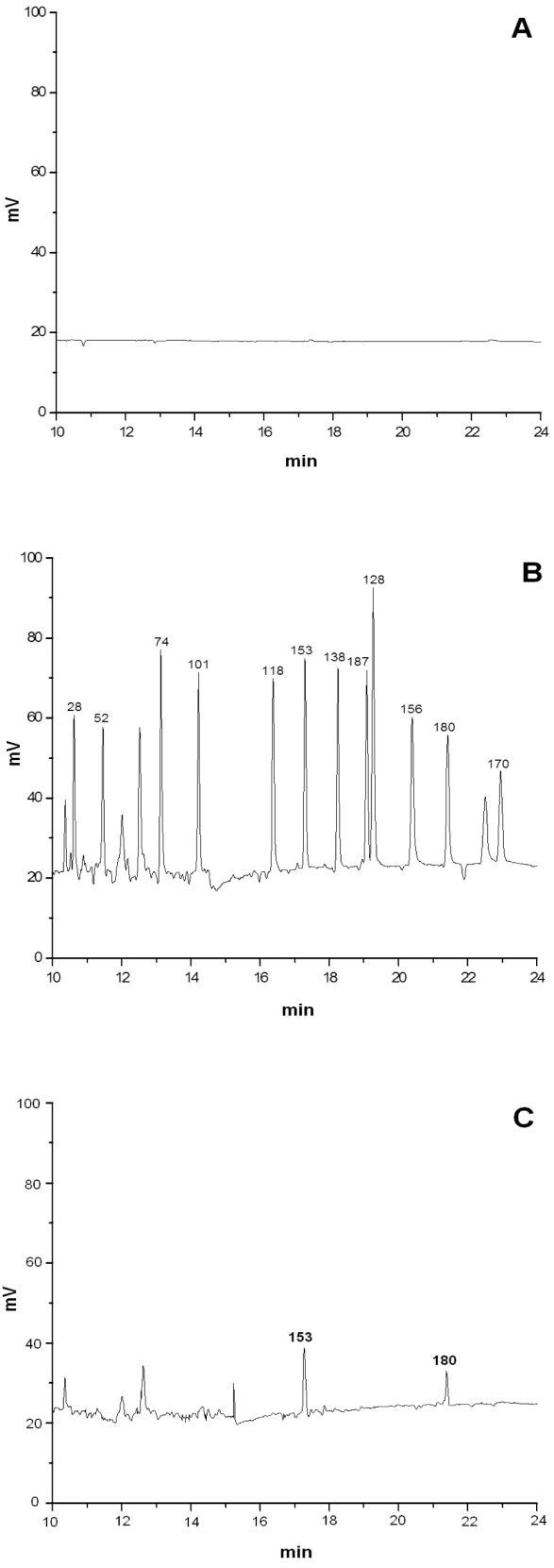

Figure 1. HS-SPME-GC-ECD chromatograms of breast milk sample. (A) blank milk sample. (B) breast milk sample spiked with 9.25 μg L^{-1} of each PCB. (C) breast milk sample containing 4.1 μg L^{-1} of PCB **153** and 1.2 μg L^{-1} of PCB **180**.

The precision, obtained by the RSD values, ranged between 4.91 (PCB **52**) and 11.76% (PCB **28**). The accuracy varied between 70.66 and 128.97% of recovery.

In order to evaluate the selectivity available and to confirm the identity of the suspected PCB present in the breast milk samples, a validation was performed in a GC-MS instrument. The same SPME experimental conditions previously defined were used. Good results were obtained according to Table 2.

Real samples analysis

Once the analytical methodology was validated, it was applied to 20 breast milk samples. In Table 3 the results obtained by HS-SPME-GC-ECD and confirmed by MS are given.

It was observed that 65% of the analyzed samples had PCB levels above LOQ. The most abundant PCB congeners found in these samples were PCB **52** (45%), PCB **74** and PCB **153** (40%), PCB **170** (20%), PCB **138** (15%), PCB **180** (10%) and **187** (5%). Considering the quantified results (> LOQ) the contamination distribution was: PCB **153** (25%), PCB **170** (20%), PCB **52** (15%), PCB **180** (10%), PCB **138** and **187** (5%). All these PCB in association with PCB **101**, PCB **118** and PCB **156** were abundantly commercialized and distributed around the world in a mixture called Aroclor® (Monsanto, USA), being the most

Table 1. Performance of the HS-SPME-GD-ECD method for PCB analysis

PCB #	Retention time / min	Linear range / (μg L^{-1})	Correlation coefficient (*r*)	Repeatability* / (%)	LOD / (μg L^{-1})	LOQ / (μg L^{-1})	Recovery / (%)				
							1 μg L^{-1}	2 μg L^{-1}	4μg L^{-1}	8 μg L^{-1}	16 μg L^{-1}
28	10.59	1-16	0.9945	11.8	0.54	1.65	71	97	82	118	86
52	11.43	1-16	0.9900	5.0	0.74	2.24	71	81	99	125	79
74	13.10	1-16	0.9884	10.3	0.80	2.42	76	97	111	126	78
101	14.19	1-16	0.9933	11.3	0.61	1.84	82	109	103	129	83
118	16.34	1-16	0.9959	5.9	0.47	1.43	89	116	101	113	87
128	19.23	0.5-16	0.9989	6.4	0.24	0.73	88	127	98	103	97
138	18.21	1-16	0.9974	6.5	0.38	1.14	96	126	101	108	91
153	17.26	1-16	0.9970	4.9	0.41	1.23	92	127	102	108	93
156	20.35	0.5-16	0.9997	8.5	0.16	0.50	92	122	96	100	100
170	22.91	0.5-16	0.9995	8.5	0.17	0.51	90	121	96	101	99
180	21.38	0.5-16	0.9996	9.1	0.15	0.45	91	120	94	99	102
187	19.05	1-16	0.9980	10.0	0.33	1.00	91	128	93	107	93

*Measure with 5 replicates of breast milk spiked with 4 μg L^{-1}.

Table 2. Performance of the HS-SPME-GD-MS method for PCB analysis

PCB #	Retention time / min	Linear range / (μg L^{-1})	Correlation coefficient (*r*)	Repeatability* / (%)	LOD / (μg L^{-1})	LOQ / (μg L^{-1})	Recovery / (%)				
							1 μg L^{-1}	2 μg L^{-1}	4μg L^{-1}	8 μg L^{-1}	16 μg L^{-1}
28	10.90	1-16	0.9959	4.27	0.79	2.41	126	112	91	83	121
52	12.07	1-16	0.9967	10.95	0.71	2.16	122	102	112	84	114
74	14.10	1-16	0.9985	4.92	0.48	1.46	132	117	88	92	110
101	15.30	1-16	0.9967	4.88	0.72	2.17	100	128	109	109	88
118	17.70	1-16	0.9965	9.97	0.73	2.22	115	121	116	105	90
128	21.26	0.5-16	0.9949	5.12	1.04	3.16	110	127	108	95	99
138	19.90	1-16	0.9941	2.53	0.96	2.91	123	123	110	94	98
153	18.80	1-16	0.9926	10.21	1.07	3.26	103	118	103	119	82
156	22.68	0.5-16	0.9929	7.40	1.23	3.74	106	121	119	95	96
170	24.10	0.5-16	0.9885	6.95	1.58	4.78	84	117	137	107	83
180	23.45	0.5-16	0.9894	10.42	1.51	4.57	93	126	117	112	82
187	21.08	1-16	0.9865	10.85	1.71	5.18	93	126	108	105	84

*Measure with 5 replicates of breast milk spiked with 4 μg L^{-1}.

Table 3. Concentration of PCB (μg L^{-1}) in the 20 breast milk samples obtained in the four studied regions

	Median	ΣPCB	PCB **28**	PCB **52**	PCB **74**	PCB **101**	PCB **118**	PCB **128**	PCB **138**	PCB **153**	PCB **156**	PCB **170**	PCB **180**	PCB **187**
São Paulo														
1	0.5	5.8	-	< LOQ*	< LOQ	-	-	-	< LOQ	4.6	-	-	-	-
2	2.3	4.7	-	< LOQ	-	-	-	-	-	4.4	-	-	-	-
3	1.1	4.8	-	< LOQ	< LOQ	-	-	-	-	3.3	-	-	-	-
4	2.7	5.3	-	-	-	-	-	-	-	4.1	-	-	1.2	-
5	3.1	13.9	-	6.2	< LOQ	-	-	-	-	4.2	-	-	1.4	-
Total	2.3	34.5	-	7.8	2.9	-	-	-	< LOQ	20.6	-	-	2.6	-
Vitória														
1	0.7	3.4	-	< LOQ	< LOQ	-	-	-	-	< LOQ	-	0.7	-	-
2	0.9	6.2	-	4.0	< LOQ	-	-	-	< LOQ	< LOQ	-	-	-	-
3	0.6	0.6	-	-	-	-	-	-	-	-	-	0.6	-	-
4	0.9	5.9	-	4.7	< LOQ	-	-	-	-	-	-	0.9	-	-
5	1.0	3.3	-	< LOQ	< LOQ	-	-	-	-	-	-	2.2	-	-
Total	0.9	19.4	-	11.1	< LOQ	-	-	-	< LOQ	< LOQ	-	4.4	-	-
Florianópolis														
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	2.1	2.1	-	-	-	-	-	-	-	-	-	-	-	2.1
4	1.0	2.0	-	-	-	-	-	-	1.7	< LOQ	-	-	-	-
5	5.9	11.8	-	10.7	< LOQ	-	-	-	-	-	-	-	-	-
Total	2.1	15.9	-	10.7	< LOQ	-	-	-	1.7	< LOQ	-	-	-	2.1
Rio Branco	-	-												
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Values below the limit of quantification and above the limit of detection (LOD < X < LOQ) were considered for the calculation of medians and sum.

common in the environment and consequently in human tissues and fluids, as breast milk. This product had been extensively used in several industrial applications, such as electrical capacitors, hydraulic fluids, adhesives and rubber plasticizers. A review study published in 2007 related that around 250.000 to 300.000 t of Aroclor still have been used in Brazil, mainly in industrial centers as São Paulo.[41] Many cases of oil leak were detected in inactive electric power stations in the state of São Paulo. This problem is yet bigger due to the difficulty to manage industrial and municipal residues. In a study performed in sewage sludge from wastewater treatment in the state of Rio de Janeiro (approximately 450 km from São Paulo) 57.6 mg kg^{-1} to the digested sludge and 145.0 mg kg^{-1} to the activated sludge were found. With regards to the indicator-PCB, a limiting value of 0.2 mg kg^{-1} of congener (d.w.) is recommended, which means that the values found in Brazilian sludge samples by far exceed this limit.[42]

All the PCB found in this study could be classified as non-dioxin-like congeners as described in the majority of the papers. Due to their higher chemical stability, PCB **153** and **180** are usually detected in breast milk samples[6,8,43] being also the most common in this study: PCB **153** was found in all samples from the city of São Paulo and PCB **180** in two of these samples. All the donors from São Paulo lived in the district of Santo Amaro and neighborhoods. In the 1970's and 1980's, this region was the primary industrial center of the city but it has recently changed with industries moving to the countryside. In 2005, the Company of Technology and Environmental Sanitation (CETESB) from São Paulo sealed off several deep wells in this region because of the confirmation of subterranean and superficial

water contamination with chlorinated substances.[44] It is still more worrying since the dam which distributes water for the whole metropolitan region is located in this area. Figure 1C shows a typical chromatogram of a São Paulo breast milk sample contaminated with 4.1 μg L^{-1} of PCB **153** and 1.2 μg L^{-1} of PCB **180**. Throughout this study, the maximum of contamination (11.78 μg L^{-1} of PCB) was found in a sample from São Paulo with 6.19 μg L^{-1} of PCB **52**, 4.18 μg L^{-1} of PCB **153** and 1.41 μg L^{-1} of PCB **180**.

All the samples collected in Vitória were contaminated by some PCB: however, the most common in these samples was PCB **170** which was quantified in 80% of the samples. Vitória is an island and two big harbors support the local economy besides steelworks and mining industries. Two of the mothers reported they lived near steelworks industries and another mother mentioned the presence of a rubber industry in the vicinity. Furthermore, the donors informed they used to eat sea fish and crustaceans at least once a week, which could be another contamination source.

Florianopolis just like Vitória, is situated on an island, although, in this case, the economy is based mainly in tourist and leisure activities, and the big industries are settled in other cities of the state. 60% of contamination was found in these samples, all of which were provided by donors from the countryside of the state of Santa Catarina. One of these samples was contaminated with 10.7 μg L^{-1} of PCB **52**: this donor informed she used to eat sea fish and crustaceans more than once a week. In addition, her family does not have water and sewage treatment in their district. Possibly, in this state, the contamination is distributed to the countryside according to the industries distribution.

In Rio Branco, no contamination at quantification levels was found. These mothers reported they used to eat fish from local rivers at least once a week. Probably, the PCB levels are lower in this region than in the others because it is located in a remote area within the Amazon region.

Conclusions

The HS-SPME-GC-ECD method developed and applied in this study proved to be a simple, fast and convenient tool for the simultaneous determination of twelve PCB congeners in breast milk samples. The use of an optimized HS-SPME condition allowed the increase in the efficiency of the proposed method. Acceptable results of linearity, precision, recovery, selectivity, LOD and LOQ were obtained in the validation process. The sample analysis revealed that no PCB was found in breast milk samples from the Amazon region. On the other hand, all samples from industrialized cities like São Paulo and Vitória showed some contamination. An extensive study will be necessary to map the whole country and, afterwards to investigate the development of these children. However, the practice of breast-feeding should not be discouraged because of well-recognized advantages.

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